PENTACYCLIC OXEPINES AND DERIVATIVES THEREOF, COMPOSITIONS AND METHODS

BACKGROUND OF THE INVENTION

The present invention relates to pentacyclic oxepines and derivatives thereof, compositions containing those compounds, their use as selective estrogen receptor modulators, and their use in inhibiting bone loss, cardiovascular disease, and breast and uterine carcinoma.

Menopause, the transition in women from the reproductive to the non-reproductive stage of life, is characterized by the cessation of menstruation and occurs at an average age of fifty years. The postmenopausal state is characterized by changes in the levels of circulating sex hormones, the most dramatic of which is the reduction in plasma levels of 17β-estradiol to less than ten percent of premenopausal values. Clinical and epidemiological studies have shown that the postmenopausal state is an important risk factor for a number of chronic disorders, notably osteoporosis and cardiovascular disease. In view of the fact that the current life span of women is about eighty years, women spend approximately one-third of their lives in the postmenopausal state. This means that the potential for chronic effects of the postmenopausal state on women's health is greater today than at the turn of the century when life expectancy was considerably shorter.

Osteoporosis describes a group of diseases which are characterized by the net loss of bone mass per unit volume. The consequence of this loss of bone mass and resulting bone fracture is the failure of the skeleton to provide adequate structural support for the body. The most vulnerable bone tissue to the effects of postmenopausal osteoporosis is the trabecular bone. This tissue is often referred to as spongy or cancellous bone and is particularly concentrated near the ends of the bone (near the joints) and in the vertebrae of the spine. The trabecular tissue is characterized by small osteoid structures which inter-connect with each other, as well as the more solid and dense cortical tissue which makes up the outer surface and central shaft of the bone. This inter-connected network of trabeculae gives lateral support to the outer cortical structure and is critical to the biomechanical strength of the overall structure.

Following the cessation of menses, most women lose from about 20% to about 60% of the bone mass in the trabecular compartment of the bone within 3 to 6 years. This rapid loss is generally associated with an increase of bone resorption and formation. However, the resorptive cycle is more dominant and the result is a net loss of bone mass.

In postmenopausal osteoporosis, it is primarily the net resorption and loss of the trabeculae which leads to the failure and fracture of bone. In light of the loss of the trabeculae in postmenopausal women, it is not surprising that the most common fractures are those associated with bones which are highly dependent on trabecular support, for example the vertebrae, the neck, and the weight bearing bones such as the femur and the fore-arm. Indeed, hip fracture, collies fractures, and vertebral crush fractures are hallmarks of postmenopausal osteoporosis.

There are an estimated 25 million women in the United States alone who are afflicted with this disease. The results of osteoporosis are personally harmful and also account for a large economic loss due its chronicity and the need for extensive and long term support (hospitalization and nursing home care). This is especially true in more elderly patients. Additionally, although osteoporosis is not generally thought of as a life threatening condition, a 20% to 30% mortality rate is related with hip fractures in elderly women. A large percentage of this mortality rate can be directly associated with postmenopausal osteoporosis.

Cardiovascular disease is the leading cause of death among women. Compared to men, premenopausal women are relatively protected from cardiovascular disease; however, this protection is gradually lost following menopause. The nature of estrogen's ability to regulate serum lipids is not well understood, but evidence indicates that estrogen can up-regulate the low density lipid (LDL) receptors in the liver which act to remove excess cholesterol. Additionally, estrogen appears to have some effect on the biosynthesis of cholesterol, and other beneficial effects on cardiovascular health.

At the present time, one generally accepted method for treatment of disorders resulting in the postmenopausal state from the decline in estrogen levels is estrogen replacement therapy. The therapy may take the form of administering estrogen alone in so-called unopposed estrogen replacement therapy (ERT) or in the form of coadministering estrogen and progestin in a so-called hormonal replacement therapy (HRT) regimen. There are, however, major liabilities associated with chronic

administration of estrogen in postmenopausal women having to do with adverse effects on the breast and uterus. Women on ERT develop endometrial cancer at rates three to six times higher than nonusers after three to six years of use; after ten years of ERT, the risk ratio increases to tenfold.

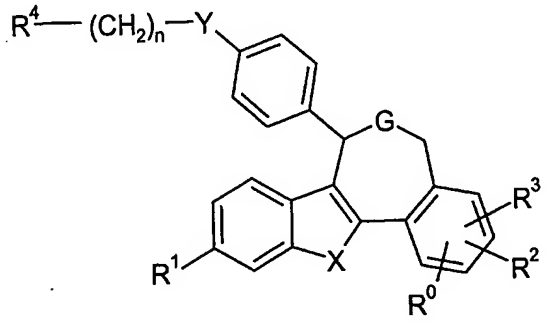
To combat these deleterious effect of ERT, the coadministration of progestin along with estrogen in a combined hormonal replacement therapy (HRT) is employed, since progestin acts to limit uterine stimulation and thus reduce the risk of uterine cancer.

Because of these known and suspected or feared liabilities of estrogen therapy, prescription of and patient compliance with chronic estrogen replacement therapy has been poor. It has been estimated that, in the United States among postmenopausal women for whom ERT or HRT has been prescribed, fewer than forty percent continue therapy beyond one year.

As a consequence, there is a need for the development of postmenopausal therapy agents which possess the ideal pharmacological profile: for example agents which produce the beneficial effects of estrogen upon skeletal tissue and the cardiovascular system without producing the adverse effects of estrogen upon the breast and the uterus. Agents possessing such an estrogen profile would reverse the effects of estrogen deficiency in certain tissues while at the same time bypassing or failing to act in tissues in which estrogen produces adverse effects. The term selective estrogen receptor modulators or "SERMs" has been applied such compounds which possess this tissue selective profile. SERMs are defined as compounds producing estrogen agonism in one or more desired target tissues such as bone, liver, etc., together with estrogen antagonism and/or minimal (i. e. clinically insignificant) agonism in reproductive tissues such as the breast or uterus.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a compound of the formula



(I)

wherein

R^1 is -H, -OH, -O(C_1-C_4 alkyl), -OCOC$_6$H$_5$, -OCO(C_1-C_6 alkyl), or -OSO$_2$(C_2-C_6 alkyl);

R^0, R^2 and R^3 are each independently -H, -OH, -O(C_1-C_4 alkyl), -OCOC$_6$H$_5$, -OCO(C_1-C_6 alkyl), -OSO$_2$(C_2-C_6 alkyl) or halo;

R^4 is 1-piperidinyl, 1-pyrrolidinyl, methyl-1-pyrrolidinyl, dimethyl-1-pyrrolidinyl, 4-morpholino, dimethylamino, diethylamino, diisopropylamino, or 1-hexamethyleneimino;

n is 2 or 3

X is –S- or -HC=CH-;

G is –O-, -S-, -SO-, SO$_2$, or –N(R^5)-, wherein R^5 is –H or C_1-C_4 alkyl; and

Y is –O-, -S-, -NH-, -NMe-, or -CH$_2$-;

or a pharmaceutically acceptable salt thereof.

In a second embodiment, the present invention provides a pharmaceutical composition comprising a therapeutically effective amount of a compound of formula (I), alone or in combination with estrogen or progestin, and a pharmaceutically acceptable carrier.

In a further embodiment, the present invention provides medical methods of employing compounds of the present invention, for alleviating symptoms of estrogen

deprivation, including bone loss, for example, osteoporosis; cardiovascular disease, for example hypertension, thrombosis and lowering serum cholesterol.

In an alternative embodiment of the medical method of the present invention, the compounds of the present invention are employed in the treatment of disease conditions associated with an aberrant physiological response to endogenous estrogen including uterine fibroid disease or uterine fibrosis, endometriosis, and estrogen dependent cancers.

In a still further embodiment, the invention relates to chemical intermediates of formulae (4), (5), (12), and (13) or pharmaceutically acceptable salts thereof.

DETAILED DESCRIPTION OF THE INVENTION

General terms used in the description of compounds herein described bear their usual meanings. For example, C_1-C_6 alkyl" refers to straight, branched, or cyclic aliphatic chains of 1 to 6 carbon atoms including moieties such as methyl, ethyl, propyl, isopropyl, butyl, n-butyl, pentyl, isopentyl, hexyl, isohexyl, cyclohexyl and the like. Likewise, "C_1-C_4 alkyl" refers to straight, branched, or cyclic aliphatic chains of 1 to 4 carbon atoms including moieties such as methyl, ethyl, propyl, isopropyl, butyl, n-butyl, cyclopropyl, and the like. Similarly, the term "C_1-C_4 alkoxy" represents a C_1-C_4 alkyl group attached through an oxygen molecule and include moieties such as, for example, methoxy, ethoxy, n-propoxy, isopropoxy, and the like.

The term "NMe" refers to methylamino. The term "halo" refers to bromo, chloro, fluoro and iodo.

As used herein, the term "stereoisomer" refers to a compound made up of the same atoms bonded by the same bonds but having different three-dimensional structures which are not interchangeable. The three-dimensional structures are called configurations. As used herein, the term "enantiomer" refers to two stereoisomers whose molecules are nonsuperimposable mirror images of one another. The term "chiral center" refers to a carbon atom to which four different groups are attached. As used herein, the term "diastereomers" refers to stereoisomers which are not enantiomers. In addition, two diastereomers which have a different configuration at only one chiral center are referred to herein as "epimers". The terms "racemate", "racemic mixture" or "racemic modification" refer to a mixture of equal parts of enantiomers.

The term "enantiomeric enrichment" as used herein refers to the increase in the amount of one enantiomer as compared to the other. A convenient method of expressing the enantiomeric enrichment achieved is the concept of enantiomeric excess, or "ee", which is found using the following equation:

$$ee = \frac{E^1 - E^2}{E^1 + E^2} X\ 100$$

wherein E^1 is the amount of the first enantiomer and E^2 is the amount of the second enantiomer. Thus, if the initial ratio of the two enantiomers is 50:50, such as is present in a racemic mixture, and an enantiomeric enrichment sufficient to produce a final ratio of 70:30 is achieved, the ee with respect to the first enantiomer is 40%. However, if the final ratio is 90:10, the ee with respect to the first enantiomer is 80%. An ee of greater than 90% is preferred, an ee of greater than 95% is most preferred and an ee of greater than 99% is most especially preferred. Enantiomeric enrichment is readily determined by one of ordinary skill in the art using standard techniques and procedures, such as gas or high performance liquid chromatography with a chiral column. Choice of the appropriate chiral column, eluent and conditions necessary to effect separation of the enantiomeric pair is well within the knowledge of one of ordinary skill in the art. In addition, the specific stereoisomers and enantiomers of compounds of formula I can be prepared by one of ordinary skill in the art utilizing well known techniques and processes, such as those disclosed by J. Jacques, et al., "Enantiomers, Racemates, and Resolutions", John Wiley and Sons, Inc., 1981, and E.L. Eliel and S.H. Wilen," Stereochemistry of Organic Compounds", (Wiley-Interscience 1994), and European Patent Application No. EP-A-838448, published April 29, 1998. Examples of resolutions include recrystallization techniques or chiral chromatography.

Some of the compounds of the present invention have one or more chiral centers and may exist in a variety of stereoisomeric configurations. As a consequence of these chiral centers, the compounds of the present invention occur as racemates, mixtures of enantiomers and as individual enantiomers, as well as diastereomers and mixtures of diastereomers. All such racemates, enantiomers, and diastereomers are within the scope of the present invention.

The terms "R" and "S" are used herein as commonly used in organic chemistry to denote specific configuration of a chiral center. The term "R" (rectus) refers to that configuration of a chiral center with a clockwise relationship of group priorities (highest to second lowest) when viewed along the bond toward the lowest priority group. The term "S" (sinister) refers to that configuration of a chiral center with a counterclockwise relationship of group priorities (highest to second lowest) when viewed along the bond toward the lowest priority group. The priority of groups is based upon their atomic number (in order of decreasing atomic number). A partial list of priorities and a discussion of stereochemistry is contained in "Nomenclature of Organic Compounds: Principles and Practice", (J.H. Fletcher, et al., eds., 1974) at pages 103-120.

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The designation " ┅┅ " refers to a bond that protrudes backward out of the plane of the page.

The designation " ~~ " refers to a bond wherein the stereochemistry is not defined.

As used herein, the term "estrogen" includes steroidal compounds having estrogenic activity such as, for example, 17β-estradiol, estrone, conjugated estrogen (Premarin®), equine estrogen 17α-ethynyl estradiol, and the like. As used herein, the term "progestin" includes compounds having progestational activity such as, for example, progesterone, norethylnodrel, nongestrel, megestrol acetate, norethindrone, and the like.

Preferred compounds of this invention include compounds of formula I wherein Y is –O-. Other preferred compounds of this invention include compounds of formula I wherein G is –O- or –S-.

Certain R^3 and R^4 groups also demonstrate preferable characteristics. For example, those compounds of formula I wherein R^4 is 1-pyrrolidinyl, 1-hexamethyleneimino, or 1-piperidinyl are preferred. A further preferred subgroup of the preferred 1-pyrrolidinyl, 1-hexamethyleneimino, or 1-piperidinyl compounds include those compounds wherein R^1, R^2, and R^3 are each independently –H, -OH or -OCH_3.

Although the free-base or acid forms of formula I compounds can be used in the methods of the present invention, it is preferred to prepare and use a pharmaceutically acceptable salt form. Thus, the compounds used in the methods of this invention form

pharmaceutically acceptable acid or base addition salts with a wide variety of organic and inorganic acids and bases, and include the physiologically acceptable salts which are often used in pharmaceutical chemistry. Such salts are also part of this invention. Typical inorganic acids used to form such salts include hydrochloric, hydrobromic, hydroiodic, nitric, sulfuric, phosphoric, hypophosphoric, and the like. Salts derived from organic acids, such as aliphatic mono and dicarboxylic acids, phenyl substituted alkanoic acids, hydroxyalkanoic and hydroxyalkandioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, may also be used. Such pharmaceutically acceptable salts thus include acetate, phenylacetate, trifluoroacetate, acrylate, ascorbate, benzoate, chlorobenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, methylbenzoate, o-acetoxybenzoate, naphthalene-2-benzoate, bromide, isobutyrate, phenylbutyrate, b-hydroxybutyrate, butyne-1,4-dioate, hexyne-1,4-dioate, caprate, caprylate, chloride, cinnamate, citrate, formate, fumarate, glycollate, heptanoate, hippurate, lactate, malate, maleate, hydroxymaleate, malonate, mandelate, mesylate, nicotinate, isonicotinate, nitrate, oxalate, phthalate, terephthalate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, propiolate, propionate, phenylpropionate, salicylate, sebacate, succinate, suberate, sulfate, bisulfate, pyrosulfate, sulfite, bisulfite, sulfonate, benzenesulfonate, p-bromophenylsulfonate, chlorobenzenesulfonate, ethanesulfonate, 2-hydroxyethanesulfonate, methanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, p-toluenesulfonate, xylenesulfonate, tartarate, and the like. Preferred salts are the hydrochloride and oxalate salts.

Typical bases used to form pharmaceutically acceptable addition salts would be inorganic bases, such as, sodium hydroxide, potassium hydroxide, alkali carbonates or bicarbonates, calcium carbonate, magnesium carbonate, and the like. Additionally, organic bases may be utilized to form addition salts, e.g., alkyl amines, such as, triethylamine, dimethylamine, i-propylamine, and the like.

The pharmaceutically acceptable acid or base addition salts are typically formed by reacting a compound of formula I with an equimolar or excess amount of acid or base. The reactants are generally combined in a mutual solvent such as diethyl ether or ethyl acetate. The salt normally precipitates out of solution within about one hour to 10 days and can be isolated by filtration or the solvent can be stripped off by conventional means.

Specific examples of compounds contemplated as falling within the scope of the

present invention include, but are not limited to the following compounds and their pharmaceutically acceptable salts:

1-{2-[4-(10-methoxy-5H,7H-6-oxa-12-thia-dibenzo[a,e]azulen-7-yl)-phenoxy]-ethyl}-piperidine;

1-{2-[4-(10-methoxy-5H,7H-6-oxa-12-thia-dibenzo[a,e]azulen-7-yl)-phenoxy]-ethyl}-pyrrolidine;

7-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-5H,7H-6-oxa-12-thia-dibenzo[a,e]azulen-10-ol;

7-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-5H,7H-6-oxa-12-thia-dibenzo[a,e]azulen-10-ol;

1-{2-[4-(8-methoxy-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-11-yl)-phenoxy]-ethyl}-piperidine;

1-{2-[4-(8-methoxy-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-11-yl)-phenoxy]-ethyl}-pyrrolidine;

11-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol;

11-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol;

11-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalene-2,8-diol;

11-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalene-2,8-diol;

2-fluoro-11-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol;

2-fluoro-11-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol;

1,2-difluoro-11-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol;

1,2-difluoro-11-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol;

1,2,3-trifluoro-11-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol;

1,2,3-trifluoro-11-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol;

1,3-difluoro-11-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol;

1,3-difluoro-11-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol;

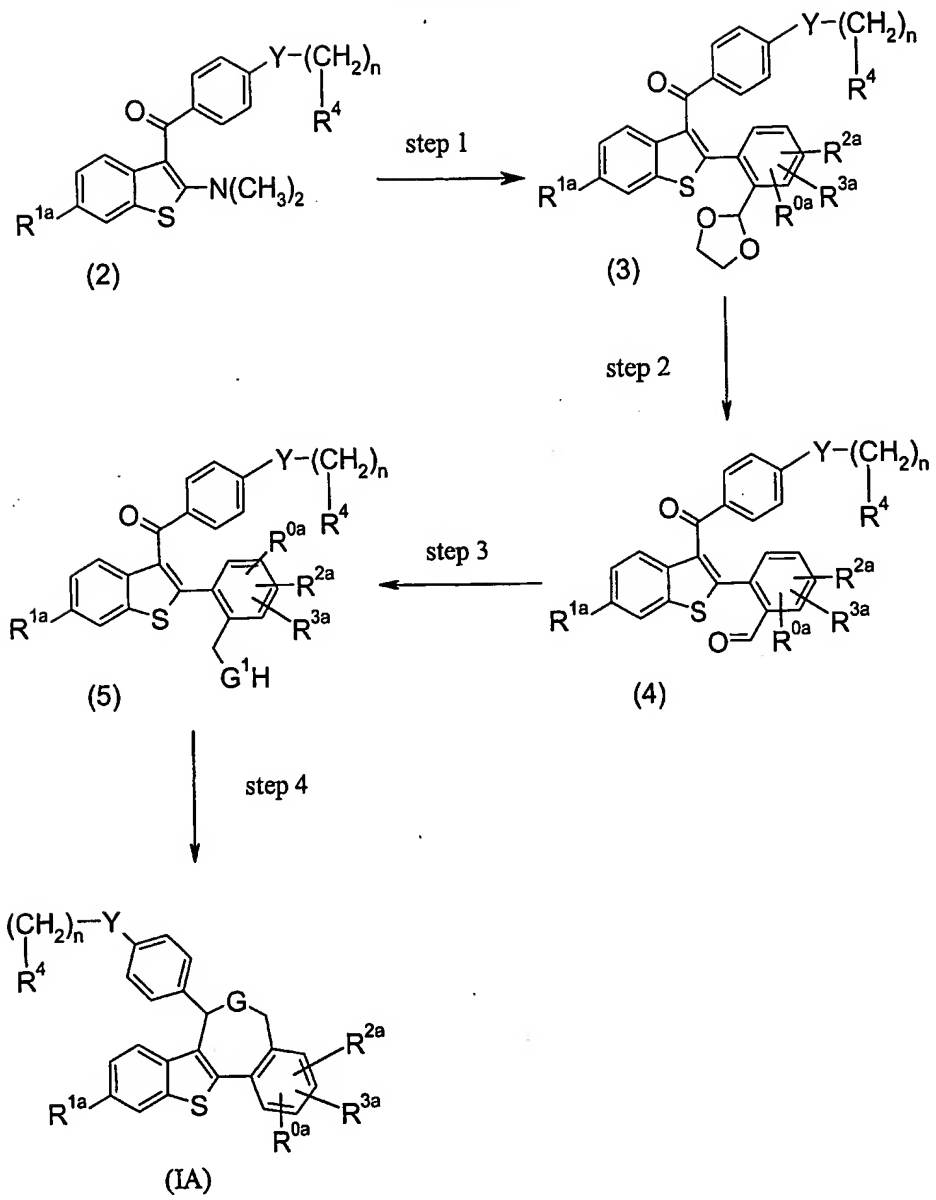
1-fluoro-11-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol;

1-fluoro-11-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol;

and pharmaceutically acceptable salts thereof.

The compounds of formula (I) can be prepared by utilizing procedures and techniques well known and appreciated by one of ordinary skill in the art. A general synthetic scheme for preparing compounds of formula (I) wherein X is –S- is set forth in Scheme A, wherein all substituents, unless otherwise indicated, are previously defined.

SCHEME A



In Scheme A, R^{0a}, R^{1a}, R^{2a}, and R^{3a} are each independently –H or –OPg, where Pg is a hydroxy protecting group and R^{0a}, R^{2a} and R^{3a} can further be halo. In compounds of formula (2), (3), et seq., the Pg protecting groups R^{0a}, R^{1a}, R^{2a}, and R^{3a} are phenolic protecting groups of the type taught by T. Greene, *et al.* in Chapter 3 of "Protective Groups in Organic Synthesis," Second Edition, John Wiley & Sons, Inc., New York,

1991, pp.143-170. The preferred protecting groups are alkyl ether groups, with methyl being particularly preferred. In Scheme A, the substituent G^1 is –O-, -S-, or $-N(R^5)-$.

In Scheme A, step 1, the 2-(2-[1,3]dioxolan-2-yl-phenyl) benzo[b]thiophene of formula (3) is prepared by reacting a dimethlyaminobenzothiophene of formula (2) with a suitable 2-(1,3-dioxolan-2-yl)phenylmagnesium bromide under Grignard conditions. The Grignard reactions are of the type taught by Godfrey (US Pat. No. 5,420,349).

For example, the dimethlyaminobenzothiophene (2) is reacted with a suitable 2-(1,3-dioxolan-2-yl)phenylmagnesium bromide under anhydrous conditions in a suitable aprotic organic solvent such as anhydrous tetrahydrofuran. The 2-(1,3-dioxolan-2-yl)phenylmagnesium is preferably present in the reaction zone in an a molar excess to dimethylaminobenzothiophene (2) of about 1.1 to about 3 equivalents. The reaction is carried out at a suitable temperature, preferably at room temperature, for a period of time ranging from about 1 to about 12 hours. The reaction is then quenched with a proton source such as, for example, sodium bicarbonate or methanol. The solvent is removed and the resulting mixture may be extracted, concentrated and purified according to techniques well known in the art, and the crude 2-(2-[1,3]dioxolan-2-yl-phenyl) benzo[b]thiophene (3) product may be used without further purification.

Appropriate dimethlyaminobenzothiophenes (2) are known in the art, Grese et al., *J. Med. Chem.* 40(2), 146-147 (1997), U.S. Pat No. 5466810 issued Nov. 14, 1995, U.S. Pat. No 5,420,349 issued May 30 1995, U.S. Pat. No. 5,792,870, issued Aug. 11, 1998, and U.S. Pat. No. 5,554,755, issued Sept. 10, 1996, or are prepared by techniques and procedures well known in the art.

In Scheme A, step 2, the benzo[b]thiophen-2-yl-benzaldehyde of formula (4) is prepared by converting the 1,3-dioxolanyl functionality of the 2-(2-[1,3]dioxolan-2-yl-phenyl) benzo[b]thiophene (3) product to an aldehyde using a suitable acid.

For example, the 2-(2-[1,3]dioxolan-2-yl-phenyl) benzo[b]thiophene (3) product is treated with a suitable acid, such as a hydrochloric acid, preferably an aqueous acid, such as a hydrochloric acid/water mixture. The reaction is carried out in a suitable organic solvent such as tetrahydrofuran or methanol. The reaction is heated to reflux for a period of time ranging from about 20 minutes to about 6 hours. The reaction is then quenched with a weak base such as, for example, sodium bicarbonate. The solvent is removed and the resulting mixture may be extracted, concentrated and purified according to techniques

well known in the art, to provide benzo[b]thiophen-2-yl-benzaldehyde (4).

In Scheme A, step 3, benzo[b]thiophen-2-yl-benzyl alcohol/thiol/amine (5) is prepared by reducing benzo[b]thiophen-2-yl-benzaldehyde (4) with a suitable reducing agent. The alcohol functionality may then be converted when a thiol or amine is desired.

For example, benzo[b]thiophen-2-yl-benzaldehyde (4) is contacted with an excess of a suitable reducing agent, such as lithium aluminum hydride (LAH), aluminum hydride, sodium borohydride, or borane dimethyl sulfide complex. The reaction is carried out in a suitable solvent, such as tetrahydrofuran or diethyl ether. The reaction is typically carried out at temperatures of from 0°C to the refluxing temperature of the solvent. Generally, the reaction requires 1 to 72 hours. For compounds where G is O, the benzo[b]thiophen-2-yl-benzyl alcohol product (5) can be isolated and purified by techniques well known in the art, such as extraction, evaporation, trituration, chromatography, and recrystallization, or alcohol of formula (5) may be used without further purification. For compounds where G is S, the alcohol of formula (5) is converted into the corresponding thiol using procedures known in the art such as initial conversion to the bromide followed by displacement with HS-/Amberlite (BANDGAR, B. P.; SADAVARTE, V. S.; Synlett 2000, (6), 908-910.). Reduction of the ketone with LAH affords the corresponding alcohol. For compounds where G is $N(R^5)$, the alcohol of formula (5) is converted to the corresponding amine using methods well known in the art, such as reaction with HN_3/PPh_3. The amine is then subjected to an intramolecular reductive amination using an acid source and reducing agent such as $NaCNBH_3$ or $Na(OAc)_3BH$. Where R^5 is not H, the nitrogen may be alkylated using an alkyl halide and base, such as KOBu-t in THF.

In Scheme A, step 4, the cyclized product of formula (IA) is prepared by subjecting benzo[b]thiophen-2-yl-benzyl alcohol/thiol/amine (5) to an acid-catalyzed cyclization.

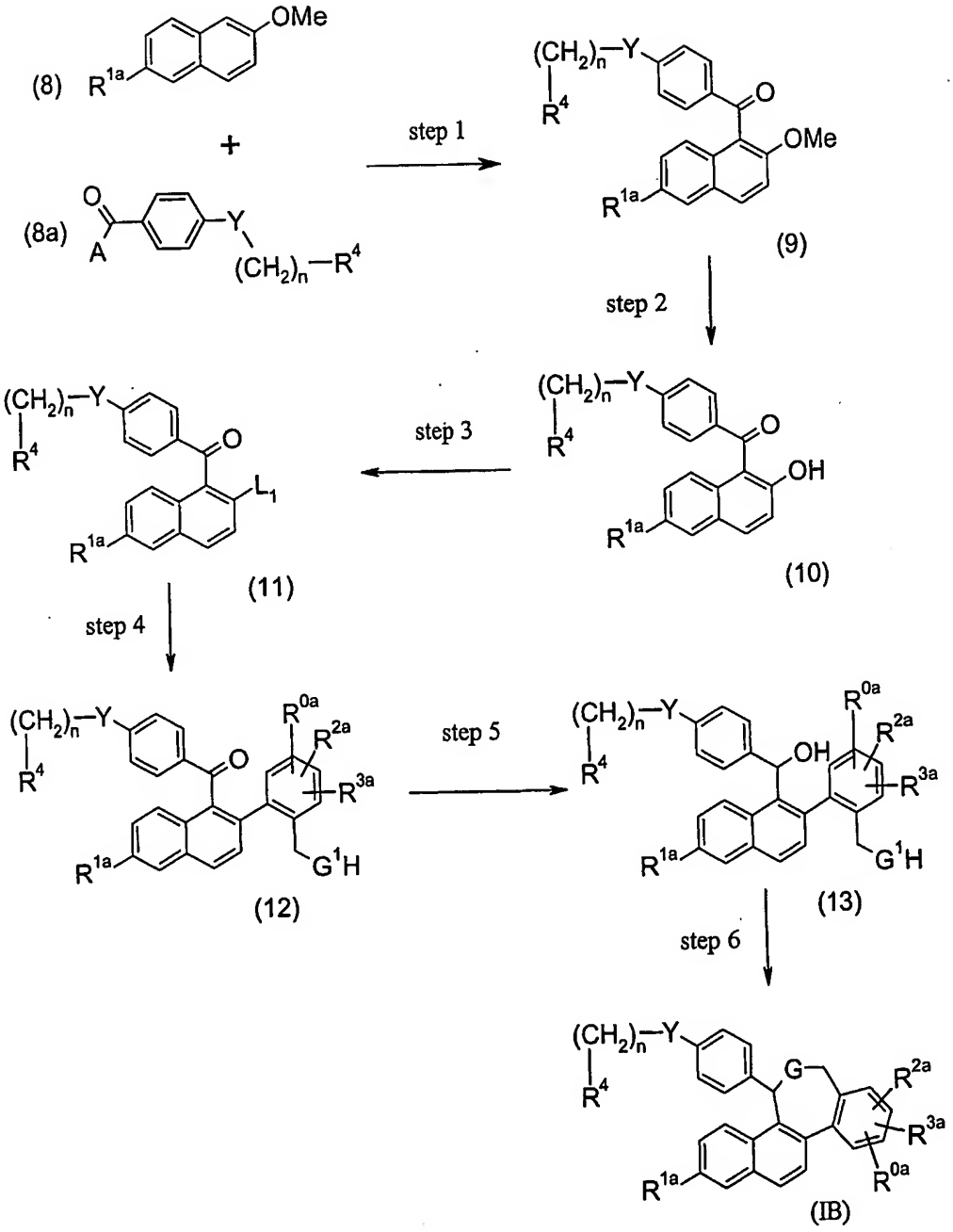
For example, benzo[b]thiophen-2-yl-benzyl alcohol/thiol/amine (5) is diluted with a suitable solvent or solvent mixture, such as tetrahydrofuran and water followed by the addition of a suitable acid, such as hydrochloric acid. The reaction mixture is then heated gently for a period of time ranging from about 5 to 30 minutes, diluted with a suitable organic solvent, such as methylene chloride, and treated with a suitable base, such as sodium carbonate, sodium bicarbonate, potassium carbonate, potassium dicarbonate,

triethylamine, or pyridine. The cyclized product of formula (IA) can be isolated and purified by techniques well known in the art, such as extraction, evaporation, trituration, chromatography, and recrystallization.

When a hydroxy group is desired at R^0, R^1, R^2, and/or R^3, a compound of formula (IA) is deprotected with a suitable deprotecting agent such as BBr_3, or sodium ethanthiolate (NaSEt). The reaction is conveniently carried out in a suitable organic solvent such as DMF, THF, or N-methylpyrrolidinone (NMP). The reaction is quenched with water and diluted with a suitable organic solvent such as methylene chloride. The deprotected product where R^0, R^1, R^2, and/or R^3 are hydroxy can be isolated and purified by techniques well known in the art, such as extraction, evaporation, trituration, chromatography, and recrystallization.

A general synthetic scheme for preparing compounds of formula (I) wherein X is –CH=CH- is set forth in Scheme B, wherein all substituents, unless otherwise indicated, are previously defined.

SCHEME B


(8)
OMe
R1a
+
(8a)
O
A
Y
(CH2)n—R4
step 1
(CH2)n—Y
R4
O
OMe
R1a
(9)
step 2
(CH2)n—Y
R4
O
OH
R1a
(10)
step 3
(CH2)n—Y
R4
O
L1
R1a
(11)
step 4
(CH2)n—Y
R4
O
R0a
R2a
R3a
G1H
R1a
(12)
step 5
(CH2)n—Y
R4
OH
R0a
R2a
R3a
G1H
R1a
(13)
step 6
(CH2)n—Y
R4
G
R2a
R3a
R0a
R1a
(IB)

5

In Scheme B, R^{0a}, R^{1a}, R^{2a}, and R^{3a} are each independently –H or –OPg, where Pg is a hydroxy protecting group and R^{0a}, R^{2a} and R^{3a} can further be halo. In compounds of formula (8), (9), et seq., the Pg protecting groups R^{0a}, R^{1a}, R^{2a}, and R^{3a} are phenolic protecting groups capable of withstanding the conditions of the Friedel-Crafts acylation reaction and are of the type taught by T. Greene, *et al.* in Chapter 3 of "Protective Groups in Organic Synthesis," Second Edition, John Wiley & Sons, Inc., New York, 1991, pp.143-170. The preferred protecting groups are alkyl ether groups, with methyl being particularly preferred. In Scheme B, the substituent G^1 is –O-, -S-, or $-N(R^5)-$.

The activating group, A, is selected from groups well known in the art to activate acids for the purposes of carrying out Friedel-Crafts acylation reactions and include the acid halides, such as fluoride, chloride and bromide; mixed acid anhydrides with C_1-C_6 alkanoic acids; C_1-C_6 alkylsulfonic acids, arylsulfonic acids, perfluorinated C_1-C_6 alkanoic acids, C_1-C_6 alkylcarbonates, arylcarbonates, and the like. The preferred compounds of formula (8a) are those in which A is halogen, most preferably chlorine.

In Scheme B, step 1, a substituted naphthyl methanone of formula (9) is prepared by conducting a Friedel-Crafts acylation of a substituted naphthyl of formula (8) with a substituted benzoyl derivative of formula (8a).

For example, the acylation reaction between (8) and (8a) is carried out in an inert organic solvent in the presence of a Lewis acid catalyst. Suitable solvents include halogenated hydrocarbons such as dichloromethane, chloroform, 1,2-dichloroethane, carbon tetrachloride, chlorobenzene, dichlorobenzene, methylene chloride, and the like. The amount of solvent is not critical, but is generally sufficient to enable efficient mixing of the reaction components. Suitable Lewis acid catalysts for the Friedel-Crafts acylation reaction between (8) and (8a) include anhydrous aluminum, boron, or zinc halides with aluminum chloride being preferred. Temperature and time of reaction will vary, depending upon the choice of reaction solvent, Lewis acid catalyst, and activating group, A. Generally, reactions are carried out at temperatures below or at ambient to below or at the reflux temperature of the solvent. Reaction times vary from several minutes to about forty-eight hours. The progress of the reaction toward completion can be followed by well-known techniques such as thin-layer chromatographic analysis of aliquots of the reaction mixture during the course of the reaction.

Typically, the reaction is conducted using 1.0 to 1.5 equivalents of compound (8a) for each equivalent of compound (8), with more of the activated benzoyl compound added during the course of the reaction as needed to drive the reaction to completion. The amount of Lewis acid catalyst employed ranges from between about 0.1 to about 5 equivalents. The substituted naphthyl methanone of formula (9) can be isolated and purified by techniques well known in the art, such as extraction, evaporation, trituration, chromatography, and recrystallization.

Appropriate substituted benzoyl derivatives of formula (8a) can be prepared as described herein from its appropriate benzoic acid derivative as set forth analogously in U.S. Pat. No. 5,962,475, the disclosure of which is hereby incorporated by reference. Appropriate benzoic acid derivatives of compounds of formula (8a) are set forth in U.S. Pat. No. 4,418,068, U.S. Pat. No. 5,631,369, and U.S. Pat. No. 5,852,193, the disclosures of which are hereby incorporated by reference.

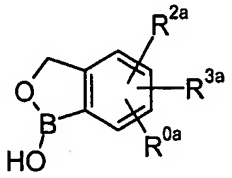
In Scheme B, step 2, the 2-hydroxy-6-substituted-naphthalen-1-yl-methanone of formula (10) is prepared by selectively deprotecting the substituted naphthyl methanone of formula (9).

For example, the naphythyl methanone of formula (9) is reacted with a demethylation reagent such as boron tribromide, boron trichloride, or boron triiodide, or with $AlCl_3$ and various thiol reagents, such as EtSH. The reaction is conducted under an inert atmosphere such as nitrogen, with one or more moles of the reagent per mole of methoxy group to be demethylated. Appropriate solvents for this reaction are those solvents or mixture of solvents which remain inert throughout the demethylation reaction. Halogenated solvents such as dichloromethane, 1,2-dichloroethane, chloroform, methylene chloride, or aromatic solvents such as benzene or toluene are preferred. The temperature employed in this reaction of the present process should be sufficient to effect completion of the demethylation reaction. However, it is advantageous to keep the temperature below 0°C in order to maximize selectivity for demethylation of the desired methoxy group and avoid the formation of undesirable by products, especially the dihydroxy analog arising from excessive demethylation. Under the preferred reaction conditions, a selectively dealkylated product will be formed after stirring the reaction for about 1 to 24 hours. After quenching the reaction, the 2-hydroxy-6-substituted-naphthalen-1-yl-methanone of formula (10) can be isolated and purified by techniques

well known in the art, such as extraction, evaporation, trituration, chromatography, and recrystallization.

In Scheme B, step 3, a 2-L-substituted-6-substituted-naphthalen-1-yl-methanone of formula (11) is prepared by converting the hydroxy group of the 2-hydroxy-6-substituted-naphthalen-1-yl-methanone of formula (10) to an appropriate activating group.

An appropriate activating group, L_1, is one which is compatible with Suzuki coupling conditions and can be displaced by a 1-hydroxy-2,1-benzoxaborolane of the formula



(11a).

Appropriate activating groups, L_1, include bromo, iodo, and most preferably trifluoromethansulfonate.

For example, compounds in which L_1 is trifluoromethansulfonate are formed by contacting an appropriate 2-hydroxy-6-substituted-naphthalen-1-yl-methanone of formula (10) with a molar excess of trifluoromethanesulfonyl chloride. The reaction is carried out in a suitable solvent, such as dichloromethane, chloroform, toluene, benzene, or pyridine. The reaction is carried out in the presence of a suitable base, such as triethylamine, diisopropylethyl amine, or pyridine. Generally the reaction is carried out at temperatures of from -20°C to 50°C. Generally, the reactions require from 30 minutes to 24 hours. The product can be isolated and purified by techniques well known in the art, such as extraction, evaporation, trituration, chromatography, and recrystallization.

In Scheme B, step 4, a [2-(2-substiuted-phenyl)-6-substituted-naphthalen-1-yl]-[4-substituted-phenyl]-methanone of formula (12) may be prepared by coupling a 2-L-substituted-6-substituted-naphthalen-1-yl-methanone of formula (11) to a 1-hydroxy-2,1-benzoxaborolane of formula (11a) using standard Suzuki coupling procedures [see, e.g., Suzuki, A., Pure and Appl. Chem., 6(2):213-222 (1994)].

For example, a slight excess of -hydroxy-2,1-benzoxaborolane of formula (11a) with each equivalent of 2-L-substituted-6-substituted-naphthalen-1-yl-methanone of formula (11) in the presence of a palladium catalyst and an appropriate base in an inert

solvent, such as toluene or 1,2-dimethoxyethane. Although various palladium catalysts drive Suzuki coupling reactions, the catalyst selected is usually reaction-specific. The use of a triphenylphosphine palladium catalyst in the present reaction is a preferred catalyst. Likewise, various bases may be used in the present coupling reaction. However, it is preferred to use Na_2CO_3. The temperature employed in this step should be sufficient to effect completion of the coupling reaction. Typically, gently heating the reaction mixture for a period from about 2 to about 8 hours is adequate. For compounds where G is O, the product (12) can be isolated and purified by techniques well known in the art, such as extraction, evaporation, trituration, chromatography, and recrystallization. For compounds where G is S or $N(R^5)$, the alcohol product of formula (12) can be converted to either the corresponding thiol or amine according to procedures set forth previously in Scheme A, step 3.

Compounds of formula (11a) are either commercially available or derived from commercially available compounds via procedures well known to one of ordinary skill in the art [see, e.g., Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, Fourth Edition, 3-16, (J. March, ed., John Wiley & Sons, Inc. 1992)].

In Scheme B, step 5, a [2-(2-substiuted-phenyl)-6-substituted-naphthalen-1-yl]-[4-substituted-phenyl]-methanol of formula (13) may be prepared by reducing a [2-(2-substiuted-phenyl)-6-substituted-naphthalen-1-yl]-[4-substituted-phenyl]-methanone of formula (12).

For example, a [2-(2-substituted-phenyl)-6-substituted-naphthalen-1-yl]-[4-substituted-phenyl]-methanone of formula (12) is reacted with an appropriate reducing agent, such as an alkali metal hydride, preferably lithium aluminum hydride. The reaction is carried out in a suitable solvent, such as tetrahydrofuran. The reaction is generally carried out at a temperature of from 0°C to the refluxing temperature of the solvent. Generally, the reactions require from 1 to 72 hours. The product (13) is preferably used without further purification, but may be isolated and purified by techniques well known in the art.

In Scheme B, step 6, the cyclized product of formula (IB) is prepared by subjecting the [2-(2-substituted-phenyl)-6-substituted-naphthalen-1-yl]-[4-substituted-phenyl]-methanol of formula (13) to an acid-catalyzed cyclization according to procedures set forth previously in Scheme A, step 4.

When a hydroxy group is desired at R^0, R^1, R^2, and/or R^3, a compound of formula (IB) can be deprotected, isolated and purified according to procedures set forth previously in Scheme A.

When a -OC(O)(C_1-C_6 alkyl) or -OC(O)C_6H_5 group is desired at R^0, R^1, R^2, and/or R^3 a mono-, di-, or trihydroxy compound of formula I, is reacted with an agent such as acyl chloride, bromide, cyanide, or azide, or with an appropriate anhydride or mixed anhydride. The reactions are conveniently carried out in a basic solvent such as pyridine, lutidine, quinoline or isoquinoline, or in a tertiary amine solvent such as triethylamine, tributylamine, methylpiperidine, and the like. The reaction also may be carried out in an inert solvent such as ethyl acetate, dimethylformamide, dimethylsulfoxide, dioxane, dimethoxyethane, acetonitrile, acetone, methyl ethyl ketone, and the like, to which at least one equivalent of an acid scavenger, such as a tertiary amine, has been added. If desired, acylation catalysts such as 4-dimethylaminopyridine or 4-pyrrolidinopyridine may be used. See, e.g., Haslam, *et al.*, Tetrahedron, 36:2409-2433 (1980).

The acylation reactions which provide the aforementioned R^0, R^1, R^2, and/or R^3 groups are carried out at moderate temperatures in the range from about -25°C to about 100°C, frequently under an inert atmosphere such as nitrogen gas. However, ambient temperature is usually adequate for the reaction.

Such acylations of the hydroxy group also may be performed by acid-catalyzed reactions of the appropriate carboxylic acids in inert organic solvents or neat. Acid catalysts such as sulfuric acid, polyphosphoric acid, methanesulfonic acid, and the like are used.

The aforementioned R^0, R^1, R^2, and/or R^3 groups also may be provided by forming an active ester of the appropriate acid, such as the esters formed by such known reagents as dicyclohexylcarbodiimide, acylimidazoles, nitrophenols, pentachlorophenol, N-hydroxysuccinimide, and 1-hydroxy-benzotriazole. See, e.g., Bull. Chem. Soc. Japan, 38:1979 (1965), and Chem. Ber., 788 and 2024 (1970).

When a compound is desired in which R^0, R^1, R^2, and/or R^3 are -OSO_2(C_4-C_6 alkyl), the suitable starting mono-, di- or trihydroxy compound is reacted with, for example, a derivative of the appropriate sulfonic acid such as a sulfonyl chloride, bromide, or sulfonyl ammonium salt, as taught by King and Monoir, J. Am. Chem. Soc.,

97:2566-2567 (1975). The mono-, di- or trihydroxy compound also can be reacted with the appropriate sulfonic anhydride. Such reactions are carried out under conditions such as were explained above in the discussion of reaction with acid halides and the like.

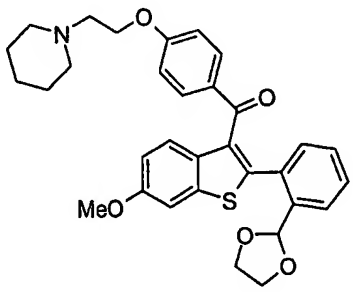
Compounds of formula (I) can be prepared so that R^0, R^1, R^2, and/or R^3 are different biological protecting groups or, preferably, the same biological protecting group. Preferred protecting groups include $-CH_3$, $-C(O)C(CH_3)_3$, $-C(O)C_6H_5$, and $-SO_2(CH_2)_3CH_3$.

Compounds of formula (8a) wherein Y is –S-, -NH-, -NMe-, or $-CH_2-$ may be prepared analogously according to procedures well known in the art. For example, preparative syntheses of compounds of formula (8a) are taught by C.R. Schmidt et al., Bioorg. Med. Chem. Lett. 9 (1999) 523-528.

All solvents were ACS grade and were used as supplied. All reagents were commercially available and used without further purification unless otherwise noted. LCMS data was recorded on a Hewlett Packard 1100 series instrument. The method used was 5% acetonitrile - 95% water (0.05% TFA) to 95% acetonitrile – 5% water (0.05% TFA) over two minutes and hold for three minutes on a Waters Symmetry C18 2.1x50mm column at 35 °C. ^1H NMR spectra were recorded at 400 MHz on a Varian 400 spectrometer unless otherwise noted.

PREPARATION 1

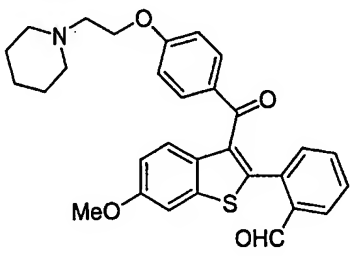
[2-(2-[1,3]Dioxolan-2-yl-phenyl)-6-methoxy-benzo[b]thiophen-3-yl]-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone



Add a solution of 2-(1,3-dioxolan-2-yl)phenylmagnesium bromide (15 mL of 0.5 M/THF, 7.5 mmol) to a stirred solution of (2-dimethylamino-6-methoxy-benzo[b]thiophen-3-yl)-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone (650 mg, 1.48 mmol) in THF (5 mL) at r.t. Stir the reaction mixture at r.t. for 2 h and quench with aq. $NaHCO_3$. Dilute with CH_2Cl_2 and wash with water and brine, dry over $MgSO_4$, filter and concentrate to afford [2-(2-[1,3]dioxolan-2-yl-phenyl)-6-methoxy-benzo[b]thiophen-3-yl]-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone. Use the product without further purification.

PREPARATION 2

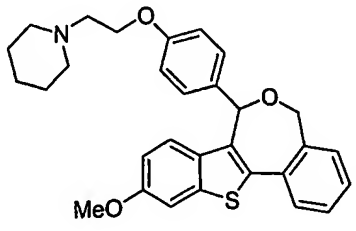
2-{6-Methoxy-3-[4-(2-piperidin-1-yl-ethoxy)-benzoyl]-benzo[b]thiophen-2-yl}-benzaldehyde



To a solution of [2-(2-[1,3]dioxolan-2-yl-phenyl)-6-methoxy-benzo[b]thiophen-3-yl]-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone (crude) in THF (15 mL), add HCl (2 mL, 5M) and water (2 mL). Heat the mixture to reflux for 30 min. Dilute with CH_2Cl_2 and quench with aq. $NaHCO_3$. Wash the organic phase with water and brine, dry over $MgSO_4$, filter and concentrate. Purify the crude product by flash chromatography (0 – 10% (2M NH_3 in MeOH)/CH_2Cl_2) to afford 2-{6-methoxy-3-[4-(2-piperidin-1-yl-ethoxy)-benzoyl]-benzo[b]thiophen-2-yl}-benzaldehyde (410 mg) which is contaminated with unidentified byproducts and is used without further purification.

EXAMPLE 1

1-{2-[4-(10-Methoxy-5H,7H-6-oxa-12-thia-dibenzo[a,e]azulen-7-yl)-phenoxy]-ethyl}-piperidine



Add LAH solution (1M/THF) to a solution of 2-{6-methoxy-3-[4-(2-piperidin-1-yl-ethoxy)-benzoyl]-benzo[b]thiophen-2-yl}-benzaldehyde (410 mg, 0.82 mmol) in THF (5 mL) at r.t. until LCMS analysis indicates complete consumption of starting material.

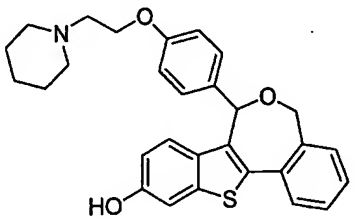
Dilute the mixture with THF (10 mL) and water (2 mL), followed by cautious addition of HCl (5 M) to pH 1. Heat gently for 10 min. Dilute with CH_2Cl_2 and quench with aq. $NaHCO_3$. Wash the organic phase with water and brine, dry over $MgSO_4$, filter and concentrate. Purify the crude product by flash chromatography (0 – 10% (2M NH_3 in MeOH)/CH_2Cl_2) to afford 1-{2-[4-(10-methoxy-5H,7H-6-oxa-12-thia-dibenzo[a,e]azulen-7-yl)-phenoxy]-ethyl}-piperidine (266 mg, 67%).

^1H NMR ($CDCl_3$, 300 MHz): 7.83 (d, J = 7.7 Hz, 1H), 7.19 – 7.40 (m, 6H), 7.11 (d, J = 9.2 Hz, 1H), 6.688 (d, J = 8.9 Hz, 1H), 6.78 (d, J = 8.8 Hz, 2H), 6.38 (s, 1H), 4.74 (ABq, 2H), 4.03 (t, J = 6.2 Hz, 2H), 3.83 (s, 3H), 2.72 (t, J = 5.9 Hz, 2H), 2.46 (br. m, 4H), 1.58 (br. m, 4H), 1.43 (br. m; 2H).

LCMS: 3.20 min; m/z = 486 $(M+H)^+$

EXAMPLE 2

7-[4-(2-Piperidin-1-yl-ethoxy)-phenyl]-5H,7H-6-oxa-12-thia-dibenzo[a,e]azulen-10-ol



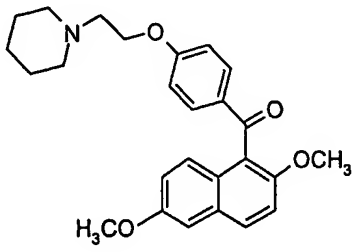
Add NaSEt (92 mg, 1.1 mmol) to a solution of 1-{2-[4-(10-methoxy-5H,7H-6-oxa-12-thia-dibenzo[a,e]azulen-7-yl)-phenoxy]-ethyl}-piperidine (266 mg, 0.55 mmol) in NMP (3 mL). Heat to 170 °C for 20 min. Add additional NaSEt (92 mg, 1.1 mmol) and continue heating for 1 h. Quench the reaction with water and dilute with CH_2Cl_2. Wash the organic phase with water and brine, dry over $MgSO_4$, filter and concentrate. Purify the crude product by flash chromatography (0 – 10% (2M NH_3 in MeOH)/CH_2Cl_2) followed by preparative HPLC to afford 7-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-5H,7H-6-oxa-12-thia-dibenzo[a,e]azulen-10-ol (16.3 mg, 6.3 %)

^1H NMR ($CDCl_3$, 300 MHz): 7.76 (d, J = 7. Hz, 1H), 7.1 – 7.4 (m, 7H), 6.98 (d, J = 8.8 Hz, 1H), 6.64 (d, J = 8.8 Hz, 2H), 6.33 (s, 1H), 4.71 (ABq, 2H), 4.05 (t, J = 5.5 Hz, 2H), 2.81 (m, 2H), 2.61 (br. s, 4H), 1.66 (m, 4H), 1.45 (br. s, 2H).

LCMS: 2.98 min; m/z = 472 $(M+H)^+$

PREPARATION 3

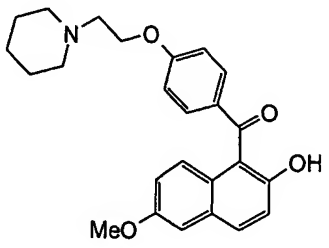
(2,6-Dimethoxy-naphthalen-1-yl)-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone



In a dry round bottom flask equipped with stir bar, temperature probe and N_2 line, dissolve 2,6-dimethoxynaphthalene (1.0 eq) in CH_2Cl_2 (5 volume equivalents) at ambient temperature. Cool the solution to 0 °C in with an ice bath, and add 4-(2-piperidin-1-yl-ethoxy)-benzoyl chloride (1.1 eq). Add aluminum chloride (2.0 eq). Once the reaction is determined to be complete, quench the reaction slowly with 1 N NaOH and dilute with additional water and CH_2Cl_2. Wash the aqueous layer with (1 x 20 mL) of CH_2Cl_2. Combine the organic extracts and wash with brine and dry (Na_2SO_4). Recrystallize the crude product from methanol to give (2,6-dimethoxy-naphthalen-1-yl)-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone (average yield 68%).

PREPARATION 4

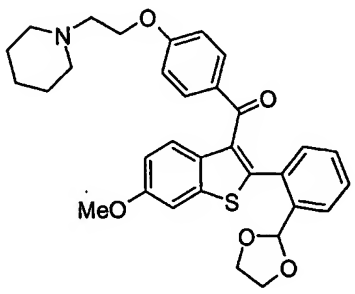
(2-Hydroxy-6-methoxy-naphthalen-1-yl)-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone



Dissolve (2,6-Dimethoxy-naphthalen-1-yl)-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone in CH_2Cl_2 (10 volume equivalents) in a 3-neck round bottom flask equipped with a pressure equalizing addition funnel, stirbar, and N_2 source. Cool the flask in an ice/brine bath and add 1.0 M BCl_3 solution in CH_2Cl_2 (1.2 equivalents) dropwise. The reaction solution turns dark red and the temperature initially increases to 5 °C. Within one hour, all starting material is consumed, as determined by TLC (1:1, Ether:Petroleum Ether). Quench the reaction with methanol (5 equivalents) and allow to warm to room temperature. Dilute the organic solution with CH_2Cl_2 (one volume equivalent) and add to a 1.0 M $NaHCO_3$ solution (5 volume equivalents) and stir for one hour. Separate the aqueous and organic layers. Wash the aqueous layer with CH_2Cl_2 (one volume) and the combine organic layers, wash with saturated NH_4Cl and dry over Na_2SO_4. Purify the product via column chromatography (50/1 silica gel) eluting with CH_2Cl_2/Hexanes (3/1) to yield (2-hydroxy-6-methoxy-naphthalen-1-yl)-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone (typical yield 87 %).

PREPARATION 5

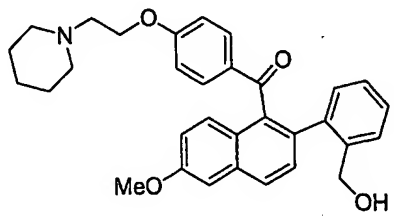
Trifluoro-methanesulfonic acid 6-methoxy-1-[4-(2-piperidin-1-yl-ethoxy)-benzoyl]-naphthalen-2-yl ester



Dissolve (2-hydroxy-6-methoxy-naphthalen-1-yl)-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone in CH_2Cl_2 (10 volumes) in a three neck round bottom flask equipped with a stir bar and N_2 source and chill to 0°C in an ice/brine bath. Add pyridine (1.3 equivalents). Add trifluoromethane sulfonyl chloride (1.2 equivalents) via syringe over 15 minutes. The yellow slurry turns clear orange with this addition. The reaction is determined to be complete by HPLC analysis after 15 minutes. Quench the reaction with H_2O (10 volumes), wash with 1 N HCl (5 volumes), wash with 1.0 N $NaHCO_3$, and dry over Na_2SO_4. After concentration, trifluoro-methanesulfonic acid 6-methoxy-1-[4-(2-piperidin-1-yl-ethoxy)-benzoyl]-naphthalen-2-yl ester as a clean yellow foam is obtained in quantitative yield. Use the product without further purification.

PREPARATION 6

[2-(2-Hydroxymethyl-phenyl)-6-methoxy-naphthalen-1-yl]-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone

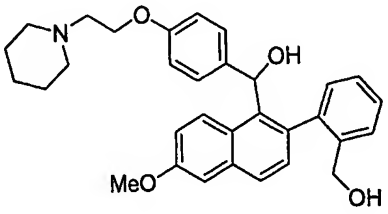


Add $Pd(OAc)_2$ (15 mg, 0.07 mmol) and Ph_3P (47 mg, 0.18 mmol) to a stirred solution of trifluoro-methanesulfonic acid 6-methoxy-1-[4-(2-piperidin-1-yl-ethoxy)-benzoyl]-naphthalen-2-yl ester (600 mg, 1.1 mmol) in DME at r.t. Add Na_2CO_3 solution (2 mL of 2 M) followed by 1-hydroxy-2,1-benzoxaborolane (200 mg, 1.5 mmol). Stir at r.t. for 2 h and heat gently (~50 °C) for 6 h. Dilute with CH_2Cl_2 and wash with water and brine, dry over $MgSO_4$, filter and concentrate. Purify the crude product by flash chromatography (0 – 5% (2M NH_3 in MeOH)/CH_2Cl_2 to yield [2-(2-hydroxymethyl-phenyl)-6-methoxy-naphthalen-1-yl]-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone (171 mg, 31%).

^1H NMR ($CDCl_3$): 7.84 (d, J = 10.2 Hz, 1H), 7.62 (br. s, 2H), 7.2 – 7.5 (m, 5H), 7.0 – 7.1 (br. m, 2H), 6.9 (br. s, 1H), 6.8 (br. m, 2H), 4.28 – 4.48 (br. m, 2H), 4.1 (br. s, 2H), 3.94 (s, 3H), 2.75 (br. m, 2H), 2.49 (br. s, 4H), 1.6 (m, 4H), 1.43 (m, 2H).

PREPARATION 7

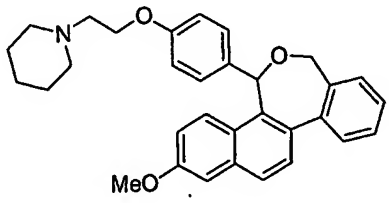
[2-(2-Hydroxymethyl-phenyl)-6-methoxy-naphthalen-1-yl]-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanol



Add LAH solution (1 mL, 1 M/THF) to a stirred solution of [2-(2-hydroxymethyl-phenyl)-6-methoxy-naphthalen-1-yl]-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone (171 mg, 0.34 mmol) in THF (5 mL) at 0 °C. Quench the reaction with sat. $NaHCO_3$ and dilute with CH_2Cl_2. Wash the organic phase with water and brine, dry over $MgSO_4$, filter and concentrate to yield [2-(2-hydroxymethyl-phenyl)-6-methoxy-naphthalen-1-yl]-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanol. Use the crude product without further purification.

EXAMPLE 3

1-{2-[4-(8-Methoxy-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-11-yl)-phenoxy]-ethyl}-piperidine



Add several drops of HCl (5M) to a stirred solution of crude [2-(2-hydroxymethyl-phenyl)-6-methoxy-naphthalen-1-yl]-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanol in THF (5 mL) and water (0.5 mL). Stir at r.t. for 24 h. Quench with aq. $NaHCO_3$ and dilute with CH_2Cl_2. Wash the organic phase with water and brine, dry over $MgSO_4$, filter and concentrate. Purify the crude product by flash chromatography (0-5% (2M NH_3/MeOH) in

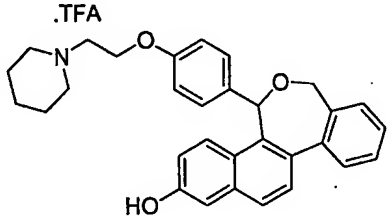
CH_2Cl_2) to yield 1-{2-[4-(8-methoxy-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-11-yl)-phenoxy]-ethyl}-piperidine (105 mg, 64 %).

^1H NMR ($CDCl_3$, 300 MHz): 8.09 (br. d, J = 9.2 Hz, 1H), 7.80 (d, J = 8.4 Hz, 1H), 7.37 (d, J = 8.8 Hz, 1H), 7.21 – 7.25 (m, 6H), 6.78 (br. s, 1H), 6.64 (br. d, J = 7.7 Hz, 2H), 6.38 (d, J = 8.8 Hz, 2H), 4.51 (ABq, 2H), 3.89 (s, 3H), 3.84 (t, J = 5.9 Hz, 2H), 2.58 (t, J = 5.9 Hz, 2H), 2.37 (br. m, 4H), 1.49 (m, 4H), 1.35 (br. m, 2H).

LCMS: 3.17 min; m/z = 480 $(M+H)^+$

EXAMPLE 4

11-[4-(2-Piperidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol trifluoroacetate



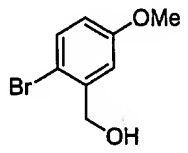
To a stirred solution of 1-{2-[4-(8-methoxy-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-11-yl)-phenoxy]-ethyl}-piperidine (40 mg, 0.08 mol) in NMP (2 mL), add NaSEt (20 mg, 0.02 mmol). Heat to reflux for 2 h and add additional NaSEt (20 mg, 0.02 mmol). After refluxing for 2 h, cool the mixture to r.t. and dilute with CH_2Cl_2. Wash the organic phase with water and brine, dry over $MgSO_4$, filter and concentrate. Purify the product by flash chromatography (0-10 % (2M NH_3/ in MeOH)/CH_2Cl_2) followed by reverse phase preparative HPLC to yield the trifluoroacetate salt of 11-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol (16 mg, 34%).

^1H NMR (CD_3OD, 400 MHz): 8.09 (br. d, J = 8.8 Hz, 1H), 7.74 (d, J = 7.8 Hz, 1H), 7.43 (d, J = 8.8 Hz, 1H), 7.23 (m, 1H), 7.1 (m, 5H), 6.81 (br. s, 1H), 6.63 (d, J = 8.3 Hz, 2H), 6.43 (d, J = 8.8 Hz, 2H), 4.57 (d, J = 11.7 Hz, 1H), 4.39 (d, J = 11.7 Hz, 1H), 4.05 (t, J = 4.9 Hz, 2H), 3.43 (br. d, J = 12.2 Hz, 2H), 3.33 (t, J = 4.9 Hz, 2H), 2.89 (br. t, J = 12.7 Hz, 2H), 1.62 – 1.85 (m, 5H), 1.42 (m, 1H).

LCMS: 2.96 min; m/z = 466 $(M+H)^+$

PREPARATION 8

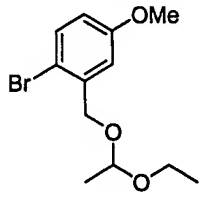
(2-Bromo-5-methoxy-phenyl)-methanol



Dissolve 2-bromo-5-methoxy-benzoic acid (2.25 g, 9.7 mmol) in THF (10 mL) and cool to 0 °C. Add 1M borane in THF (48 mL, 48 mmol) and warm to room temperature. Upon completion, slowly pour reaction onto ice and saturated aqueous sodium bicarbonate. Extract with ethyl acetate and wash organic layer with water and saturated aqueous sodium bicarbonate. Dry with sodium sulfate, filter and concentrate in vacuo to yield 2.1 g (100%) of the title compound. ^{1}H NMR ($CDCl_3$) δ7.42 (d, J = 8.8 Hz, 1H), 7.06 (d, J = 3.1 Hz, 1H), 6.72 (dd, J = 8.8, 3.1 Hz, 1H), 4.71 (s, 2H), 3.81 (s, 3H).

PREPARATION 9

1-Bromo-2-(1-ethoxy-ethoxymethyl)-4-methoxy-benzene

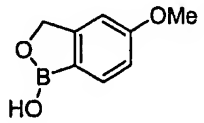


Dissolve (2-bromo-5-methoxy-phenyl)-methanol (5.0 g, 23 mmol) in dry dichloromethane (120 mL) and cool to 0 °C under nitrogen. Add ethyl vinyl ether (2.5 g, 34 mmol) followed by pyridinium *p*-toluenesulfonate (580 mg, 2.3 mmol). Warm to room temperature and stir for 2 hours. Pour reaction into saturated aqueous sodium bicarbonate and extract twice with dichloromethane. Combine organic layers and wash with water and brine. Dry with sodium sulfate, filter and concentrate in vacuo to yield 6.6 g (100%) of title compound as a clear colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.41 (d, J = 8.6 Hz, 1H), 7.08

(d, J = 3.1 Hz, 1H), 6.71 (dd, J = 8.8, 3.1 Hz, 1H), 4.88 (q, J = 5.3 Hz, 1H), 4.65 (ab, J_{ab} = 13.3 Hz, H), 4.55 (ab, J_{ab} = 13.3 Hz, 1H), 3.8 (s, 3H), 3.71 (m, 1H), 3.55 (m, 1H), 1.41 (d, J = 5.3 Hz, 3H), 1.23 (t, J = 7.0 Hz, 3H).

PREPARATION 10

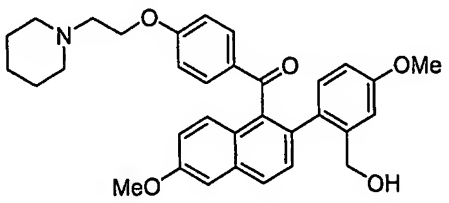
5-Methoxy-3H-benzo[c][1,2]oxaborol-1-ol



Dissolve 1-bromo-2-(1-ethoxy-ethoxymethyl)-4-methoxy-benzene (3.0 g, 10.3 mmol)in THF (100 mL) and cool to –78 °C. Add 2.5M butyllithium in hexanes (4.6 mL, 11.4 mmol), dropwise and stir for 15 min. Add trimethylborate (2.1 g, 20.6 mmol) to the reaction mixture and warm to room temperature. Add 1N HCl (100 mL) and stir at room temperature for 2 hours. Extract three times with methylene chloride, dry the combined organic layers with sodium sulfate, filter and concentrate in vacuo to yield a crude product. Triturate with methylene chloride/hexanes to yield 680 mg (40%) of the title compound. ^{1}H NMR ($CDCl_3$) δ 7.65 (d, J = 8.2 Hz, 1H), 6.93 (dd, J = 8.2, 2.1 Hz, 1H), 6.85 (d, J = 1.4 Hz, 1H), 5.06 (s, 2H), 3.86 (s, 3H).

PREPARATION 11

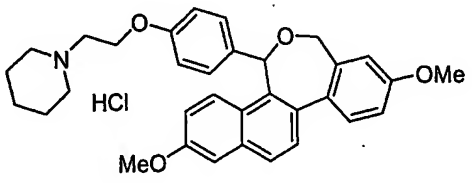
[2-(2-Hydroxymethyl-4-methoxy-phenyl)-6-methoxy-naphthalen-1-yl]-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone.



Dissolve trifluoro-methanesulfonic acid 6-methoxy-1-[4-(2-piperidin-1-yl-ethoxy)-benzoyl]-naphthalen-2-yl ester (300 mg, 0.56 mmol), 5-methoxy-3H-benzo[c][1,2]oxaborol-1-ol(275 mg, 1.7 mmol) and 2M sodium carbonate (0.5 mL, 13 mmol) in dimethoxyethane (5.0 mL). Degas the solution with nitrogen for 15 min. Add tetrakis(triphenylphosphine)palladium (130 mg, 0.11 mmol) and heat at 60 °C for 2 hours. Cool reaction to room temperature and purify on a SCX column to yield 294 mg (100%) of the title compound as a pale yellow foam. ^{1}H NMR ($CDCl_3$) 7.84 (d, J = 8.4 Hz, 1H), 7.67 (m, 2H), 7.48 (m, 1H), 7.39 (m, 1H), 7.32 (d, J = 8.4 Hz, 1H), 7.23 (d, J = 2.5 Hz, 1H), 7.07 (d, J = 8.8 Hz, 1H), 7.03 (bs, 1H), 6.82 (m, 2H), 6.57 (m, 1H), 4.44 (m, 1H), 4.28 (bs, 1H), 4.15 (bs, 2H), 3.94 (s, 3H) 3.78 (s, 3H), 2.81 (bs, 2H), 2.52 (bs, 4H) 1.61 (m, 4H), 1.44 (bs, 2H).

EXAMPLE 5

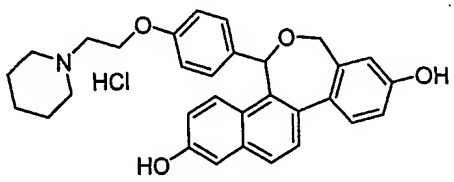
1-{2-[4-(2,8-Dimethoxy-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-11-yl)-phenoxy]-ethyl}-piperidine hydrochloride salt



Dissolve [2-(2-hydroxymethyl-4-methoxy-phenyl)-6-methoxy-naphthalen-1-yl]-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone (318 mg, 0.60 mmol) in THF (20 mL) and cool to 0 °C. Add 1M lithium aluminum hydride in THF (2.4 mL, 2.4 mmol) dropwise and warm to room temperature. Heat at reflux for 30 min. Cool to room temperature and pour onto ice/chloroform. Add 6M HCl dropwise to lower pH to 1. Extract with 20% isopropyl alcohol in chloroform. Wash organic layer with brine, dry, filter and concentrate in vacuo. Purify crude product by SCX column to yield 208 mg of a mixture of [2-(2-hydroxymethyl-4-methoxy-phenyl)-6-methoxy-naphthalen-1-yl]-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanol and the title compound. Redissolve the mixture in THF (3 mL) and add 4N HCl in dioxane (2 mL) and stir at room temperature for 1.5 hours. Partion the reaction between water and dichloromethane. Wash the organic layer with brine, dry with sodium sulfate, filter and concentrate in vacuo to yield 195 mg (64%) of the title compound. ^{1}H NMR ($CDCl_3$) δ 8.15 (d, J = 11.5 Hz, 1H), 7.86 (d, J = 8.6 Hz, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.24 (s, 1H) 7.21 (d, J = 8.8 Hz, 2H), 6.89 (d, J = 2.5 Hz, 1H), 6.79 (m, 3H), 6.46 (d, J = 8.4 Hz, 2H), 4.59 (ab, J_{ab} = 11.3 Hz, 1H), 4.51 (ab, J_{ab} = 11.3 Hz, 1H), 4.37 (s, 2H), 3.97 (s, 3H), 3.84 (s, 3H), 3.55 (m, 2H), 3.27 (s, 2H), 2.76 (m, 2H), 2.27 (m, 2H), 1.86 (m, 4H). LCMS mass spectrum (ion spray): m/z = 510.3 (M+H-HCl).

EXAMPLES 6 AND 7

11-[4-(2-Piperidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalene-2,8-diolhydrochloride salt.

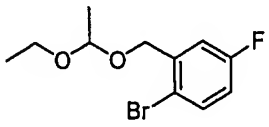


Dissolve 1-{2-[4-(2,8-dimethoxy-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-11-yl)-phenoxy]-ethyl}-piperidine (195 mg, 0.383 mmol) in DMF. Add sodium ethanethiol (306 mg, 3.65 mmol) and reflux. Reflux the reaction for 48 hours. Add more sodium ethanethiol (230 mg) and reflux for an additional 10 hours. Pour reaction onto ice and partion between water and ethyl acetate. Bring aqueous layer to a pH of about 1-2 using 6M HCl. Extract the aqueous layer three times with ethyl acetate. Combine extracts and wash with brine. Concentrate in vacuo and purify on a SCX column to yield 62mg of free base title compound. Extract the aqueous layer again to retrieve more product. Combine the products and purify by precipitation from ether. Filter the solid and redissolve with methanol and 1M HCl. Concentrate in vacuo to yield 140 mg of the title compound. ^{1}H NMR (d_6-DMSO) δ 10.87 (s, 1H), 10.02 (s, 1H), 9.71 (s, 1H), 7.79 (d, J = 8.8 Hz, 1H), 7.46 (d, J = 8.6 Hz, 1H), 7.24 (d, J = 2.1 Hz , 1H), 7.15 (m, 1H), 6.83 (d, J = 1.8 Hz, 2H), 6.7 (m, 4H), 6.58 (m, 3H), 4.50 (ab, J_{ab} = 12.5 Hz, 1H), 4.32 (ab, J_{ab} = 11.3 Hz, 1H), 4.24 (s, 2H), 3.32 (m, 4H), 2.90 (m, 2H). LCMS mass spectrum (ion spray): m/z = 482.3 (M+H-HCl).

Separate the enantiomers by chiral chromatography to yield Example 6 and Example 7.

PREPARATION 12

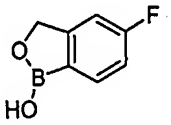
1-Bromo-2-(1-ethoxy-ethoxymethyl)-4-fluoro-benzene



Dissolve commercially available (2-bromo-5-fluoro-phenyl)-methanol (4.8 g, 23.4 mmol) in methylene chloride (100 mL) and cool to 0 °C. Add PPTS (590 mg, 2.3 mmol) followed by ethyl vinyl ether (3.4 mL, 35.1 mmol). Warm reaction to room temperature slowly after addition. After 4 hours, pour the reaction into saturated aqueous sodium bicarbonate and extract with methylene chloride. Dry with sodium sulfate, filter and concentrate in vacuo to yield 5.4 g (83%) of the title compound as a clear and colorless oil. ^{1}H NMR ($CDCl_3$) δ7.47 (dd, J = 8.6, 5.0 Hz, 1H), 7.27 (dd, J = 9.6, 3.1 Hz, 1H), 6.87 (td, J = 8.0, 3.1 Hz, 1H), 4.89 (q, J = 5.5 Hz, 1H), 4.65 (d, J = 13.8 Hz, 1H), 4.54 (d, J = 13.8 Hz, 1H), 3.70 (m, 1H), 3.55 (m, 1H), 1.42 (d, J = 5.5 Hz, 3H), 1.22 (t, J = 7.2 Hz, 3H).

PREPARATION 13

5-Fluoro-3H-benzo[c][1,2]oxaborol-1-ol

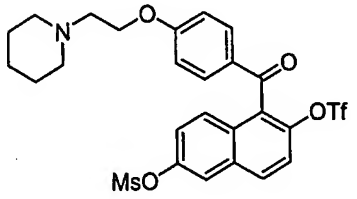


Dissolve 1-bromo-2-(1-ethoxy-ethoxymethyl)-4-fluoro-benzene(5.4g, 19.5 mmol) in dry THF (100 mL) and cool to –78 °C under nitrogen. Add butyl lithium (2.5M in Hexanes, 10.2 mL, 25.4 mmol) dropwise at –78 °C. Upon complete addition, stir the reaction at –78 °C for 10 minutes and then add trimethyl borate (4.4 mL, 39 mmol) and warm the reaction to room temperature. Pour the reaction into 1N HCl (100 mL) and stir for 1 hour. Extract the biphasic mixture with ether three times. Dry the combined organic layers with sodium sulfate, filter and concentrate in vacuo. Triturate the oily residue with

cold hexanes to yield 2.1 g (70%) of the title compoud as a white solid. ^{1}H NMR (d_6-DMSO) □9.18 (s, 1H), 7.70 (dd, J = 8.2, 5.8 Hz, 1H), 7.20 (dd, J = 9.5, 2.7 Hz, 1H), 7.11 (m, 1H), 4.92 (s, 1H).

PREPARATION 14

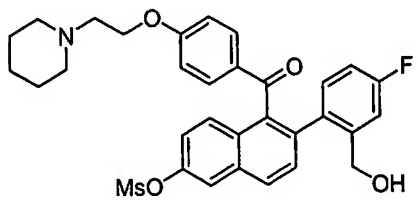
Trifluoro-methanesulfonic acid 6-methanesulfonyloxy-1-[4-(2-piperidin-1-yl-ethoxy)-benzoyl]-naphthalen-2-yl ester



Suspend trifluoro-methanesulfonic acid 6-hydroxy-1-[4-(2-piperidin-1-yl-ethoxy)-benzoyl]-naphthalen-2-yl ester (12.5 g, 24 mmol) in dry methylene chloride (100 ml). Add diisopropylethylamine (8.3 mL, 48 mmol). Slowly add methanesulfonyl chloride (2.7 mL, 36 mmol). Pour the reaction into saturated aqueous sodium bicarbonate after 20 minutes and extract with methylene chloride. Wash the organic layer with water, dry with sodium sulfate, filter and concentrate in vacuo to yield 14.2 g (99%) of the title compound as an amber foam. ^{1}H NMR ($CDCl_3$) δ8.05 (d, J = 9.2 Hz, 1H), 7.90 (d, J = 1.9 Hz, 1H), 7.76 (d, J = 8.2 Hz, 2H), 7.75 (d, J = 9.0 Hz, 1H), 7.59 (d, J = 9.2 Hz, 1H), 7.43 (dd, J = 9.2, 1.7 Hz, 1H), 6.94 (d, J = 8.2 Hz, 2H), 4.17 (t, J = 5.7 Hz, 2H), 3.23 (s, 3H), 2.79 (t, J = 5.2 Hz, 2H), 2.5 (bs, 4H), 1.63-1.57 (m, 4H), 1.48-1.40 (m, 2H); LCMS mass spectrum (ion spray): m/z = 602.3 (M+H).

PREPARATION 15

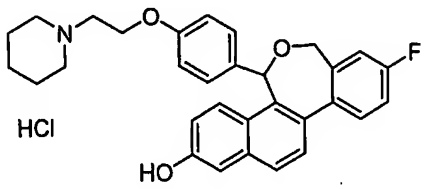
Methanesulfonic acid 6-(4-fluoro-2-hydroxymethyl-phenyl)-5-[4-(2-piperidin-1-yl-ethoxy)-benzoyl]-naphthalen-2-yl ester



Charge a 100 mL round bottom flask with trifluoro-methanesulfonic acid 6-methanesulfonyloxy-1-[4-(2-piperidin-1-yl-ethoxy)-benzoyl]-naphthalen-2-yl ester (2.3 g, 3.8 mmol), 5-fluoro-3H-benzo[c][1,2]oxaborol-1-ol (1.16 g, 7.6 mmol) and $Pd(PPh_3)_4$ (220 mg, 0.19 mmol) and purge with nitrogen. Add degassed DME (36 mL) and 2M Na_2CO_3 (4 mL) to the flask and plunge into a 75 °C oil bath and stir overnight. Pour the reaction into saturated aqueous sodium bicarbonate and extract with methylene chloride. Wash the organic layer with water and dry with sodium sulfate. Filter and concentrate to an amber foam. Purify the crude material on silica gel (1% to 6% methanol in methylene chloride) to yield 1.6 g (73%) of the title compound as a tan foam. ^1H NMR ($CDCl_3$) δ7.98 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 2.5 Hz, 1H), 7.60 (bs, 3H), 7.43 (d, J = 8.4 Hz, 1H), 7.35 (d, J = 8.6 Hz, 1H), 7.23 (bs, 1H), 6.90-6.70 (m, 4H), 4.40-4.25 (m, 2H), 4.12 (m, 2H), 3.23 (s, 3H), 2.76 (t, J = 5.4 Hz, 2H), 2.49 (bs, 4H), 1.59 (m, 5H), 1.45 (m, 1H). LCMS mass spectrum (ion spray): m/z = 600.2 (M+Na).

EXAMPLES 8 AND 9

2-Fluoro-11-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol hydrochloride salt



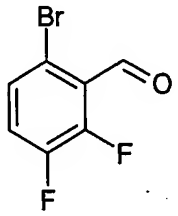
Dissolve methanesulfonic acid 6-(4-fluoro-2-hydroxymethyl-phenyl)-5-[4-(2-piperidin-1-yl-ethoxy)-benzoyl]-naphthalen-2-yl ester (1.6 g, 2.8 mmol) in dry THF (30 mL). Add lithium aluminium hydride (1M in THF, 8.3 mL, 8.3 mmol) slowly. Add THF (30 mL) after 2 hr to dissolve the precipitate. After 8 hr, add saturated NH_4Cl dropwise to quench excess $LiAlH_4$. Pour the reaction into saturated aqueous ammonium chloride and extract with methylene chloride. Wash the organic layer with water and dry with sodium sulfate. Filter and concentrate in vacuo to yield 1.4 g (100%) of a tan foam. LCMS (m/z = 502.3 (M+H))(100%). Suspend the foam in THF (30 mL) and add HCl (4N in dioxane, 3 mL, 12 mmol). After 1 hr dissolve the suspension. Stir the reaction overnight. Reduce the solvent to ~10 mL and add the resultant solution dropwise to vigorously stirred ether (100 mL). Filter the resultant white precipitate and dry in a vacuum oven overnight at 50 °C to yield 1.3 g (90%) of the title compound as a tan solid. ^1H NMR (d_6-DMSO) δ10.19 (bs, 1H), 9.94 (s, 1H), 8.24 (d, J = 9.4 Hz, 0H), 7.81 (d, J = 8.6 Hz, 1H), 7.45 (d, J = 8.6 Hz, 1H), 7.27 (m, 2H), 7.22 (d, J = 2.5 Hz, 1H), 7.13 (dd, J = 9.4, 2.5 Hz, 1H), 7.05 (td, J = 8.8, 2.7 Hz, 1H), 6.88 (bs, 1H), 6.61 (d, J = 8.4 Hz, 2H), 6.53 (d, J = 9.2 Hz, 2H), 4.63 (d, J = 11.9 Hz, 1H), 4.26 (d, J = 11.7 Hz, 1H), 4.14 (t, J = 5.6 Hz, 2H), 3.33 (m, 4H), 2.85 (m, 2H), 1.72-1.54 (m, 5H), 1.28 (m, 1H); mass spectrum (ion spray): m/z = 484.2 (M-Cl); Analysis calculated for $C_{31}H_{31}ClFNO_3 \bullet 1.20H_2O$: C, 68.74; H, 6.22; N, 2.59. Found: C, 68.74; H, 6.46; N, 2.50. Separate the enantiomers by chiral chromatography to yield Example 7 and Example 8.

General Method for the Formulation of Fluorinated Aryl Rings

Dissolve aryl bromide (1.0 eq) in dry THF (0.2 M) and cool the reaction to –78 °C under nitrogen. Add LDA (2.0 M in THF, 1.1 eq.) dropwise over 15 minutes. Stir for 20 minutes after addition complete. Add DMF (1.2 eq.) and stir at –78 °C for 15 minutes. Add glacial acetic acid (4.0 eq) followed immediately by water (12x acetic acid volume) and warm the reaction to room temperature. Separate the phases and extract the aqueous phase with ethyl acetate twice. Wash the combined organic layers with dilute aqueous HCl, water and brine. Dry with sodium sulfate, filter and concentrate. Purify the resulting residue on silica gel, eluting with 0 to 5% ethyl acetate in hexanes.

PREPARATION 16

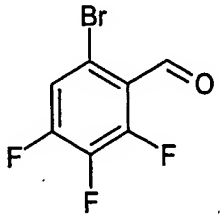
6-Bromo-2,3-difluoro-benzaldehyde



(83%) Yellow oil. ^{1}H NMR ($CDCl_3$) δ10.31 (t, J = 1.4 Hz, 1H), 7.45 (ddd, J = 9.0, 4.1, 1.9 Hz, 1H), 7.29 (td, J = 9.2, 8.0 Hz, 1H).

PREPARATION 17

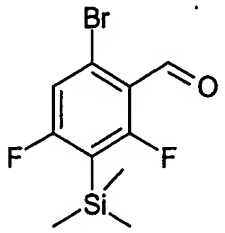
6-Bromo-2,3,4-trifluoro-benzaldehyde



(65%) Pale yellow solid. ^{1}H NMR ($CDCl_3$) δ10.24 (t, J = 1.4 Hz, 1H), 7.39 (ddd, J = 8.8, 6.2, 2.3 Hz, 1H).

PREPARATION 18

6-Bromo-2,4-difluoro-3-trimethylsilanyl-benzaldehyde



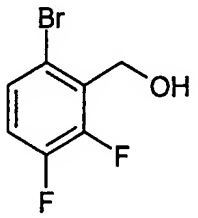
(73%) Pale yellow oil. ^{1}H NMR ($CDCl_3$) δ10.29 (d, J = 1.4 Hz, 1H), 7.17 (dd, J = 7.8, 1.6 Hz, 1H), 0.39 (s, 9H).

General Method for the Reduction of Aryl Aldehyde

Dissolve the aryl aldehyde (1.0 eq.) in methanol (0.2 M) and add sodium borohydride (2.0 eq.) portionwise. After the exothermic reaction cools to room temperature, pour the reaction into saturated aqueous ammonium chloride and extract three times with methylene chloride. Wash the combined organic layers with water and brine. Dry over sodium sulfate, filter and concentrate in vacuo to yield the title compound.

PREPARATION 19

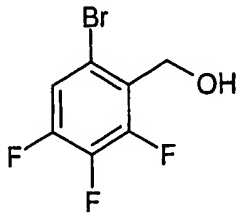
(6-Bromo-2,3-difluoro-phenyl)-methanol



(90%) White solid. ^{1}H NMR ($CDCl_3$) δ7.34 (ddd, J = 9.0, 4.5, 2.1 Hz, 1H), 7.05 (td, J = 9.3, 8.1 Hz, 1H), 4.85 (d, J = 2.3 Hz, 2H), 2.10 (bs, 1H).

PREPARATION 20

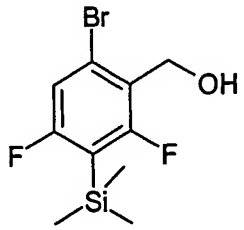
(6-Bromo-2,3,4-trifluoro-phenyl)-methanol



(100%) White solid. ^{1}H NMR ($CDCl_3$) δ7.28 (ddd, J = 9.2, 6.6, 2.5 Hz, 1H), 4.82 (dd, J = 6.6, 2.4 Hz, 2H), 2.06 (t, J = 6.6 Hz, 1H).

PREPARATION 21

(6-Bromo-2,4-difluoro-3-trimethylsilanyl-phenyl)-methanol



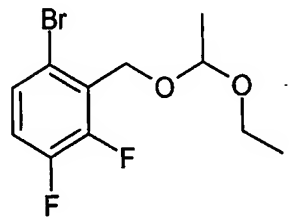
(100%) Pale yellow oil. ^{1}H NMR ($CDCl_3$) δ7.08 (dd, J = 8.0, 1.8 Hz, 1H), 4.8 (dd, J = 6.6, 2.1 Hz, 2H), 1.96 (t, J = 6.7 Hz, 1H), 0.36 (s, 9H).

Protection of Benzyl Alcohol with Ethyl Vinyl Ether

Dissolve the benzylic alcohol (1.0 eq.) in dry methylene chloride (0.2 M) under nitrogen. Cool the reaction to 0 °C and add ethyl vinyl ether (1.5 eq.) followed by pyridinium *p*-toluenesulfonate (0.1 eq.). Warm the reaction to room temperature and pour into saturated aqueous sodium bicarbonate after 2 hours. Extract with methylene chloride and wash the organic phase with water and brine. Dry over sodium sulfate, filter and concentrate in vacuo to yield the title compound.

PREPARATION 22

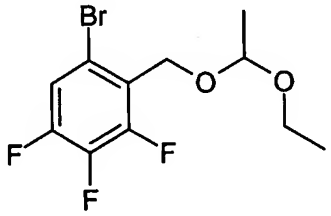
1-Bromo-2-(1-ethoxy-ethoxymethyl)-3,4-difluoro-benzene



(93%) Amber oil. ^{1}H NMR ($CDCl_3$) δ7.34 (ddd, J = 9.0, 4.5, 2.1 Hz, 1H), 7.04 (q, J = 8.8 Hz, 1H), 4.87 (q, J = 5.4 Hz, 1H), 4.79-4.65 (abx, J_{ab} = 10.5, J_{ax} = 2.7, J_{bx} = 2.7 Hz, 2H), 3.72 (m, 1H), 3.54 (m, 1H), 1.4 (d, J = 5.3 Hz, 3H), 1.23 (t, J = 7.0 Hz, 3H).

PREPARATION 23

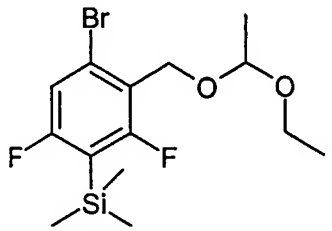
1-Bromo-2-(1-ethoxy-ethoxymethyl)-3,4,5-trifluoro-benzene



(100%) Amber oil. ^{1}H NMR ($CDCl_3$) δ7.28 (ddd, J = 9.3, 6.6, 2.5 Hz, 1H), 4.85 (q, J = 5.39 Hz, 1H), 4.75-4.60 (abx, J_{ab} = 10.7, J_{ax} = 2.9, J_{bx} = 2.7 Hz, 2H), 3.70 (m, 1H), 3.54 (m, 1H), 1.39 (d, J = 5.5 Hz, 3H), 1.23 (t, J = 7.0 Hz, 3H).

PREPARATION 24

[4-Bromo-3-(1-ethoxy-ethoxymethyl)-2,6-difluoro-phenyl]-trimethyl-silane



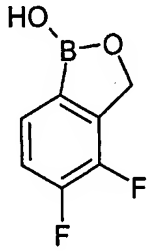
(100%). Clear colorless oil. ^{1}H NMR ($CDCl_3$) δ7.08 (dd, J = 8.0, 1.5 Hz, 1H), 4.85 (q, J = 5.4 Hz, 1H), 4.73-4.57 (abx, J_{ab} = 10.5, J_{ax} = 2.5, J_{bx} = 2.5 Hz, 2H), 3.72 (m, 1H), 3.53 (m, 1H), 1.39 (d, J = 5.5 Hz, 3H), 1.22 (t, J = 7.1 Hz, 3H), 0.35 (s, 9H).

General Method for the Formation of Hemiboronic Ester

Dissolve the aryl bromide from above (1.0 eq.) in dry THF (0.2 M) and cool to –78 °C under nitrogen. Add butyl lithium (2.5M in Hexanes, 1.1 eq.) dropwise at –78 °C. Upon complete addition, stir the reaction at –78 °C for 10 minutes and then add trimethyl borate (2.0 eq.) and warm the reaction to room temperature. Add 1N HCl (100 mL) and stir for 1 hour. Extract the biphasic mixture with ethyl acetate. Wash with water. Dry the organic layer with sodium sulfate, filter and concentrate in vacuo.

PREPARATION 25

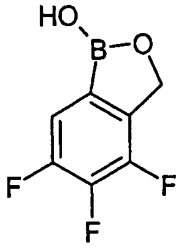
4,5-Difluoro-3H-benzo[c][1,2]oxaborol-1-ol



Used crude without purification. ^{1}H NMR (d_6-DMSO) δ9.47 (bs, 1H), 7.56 (dd, J = 7.8, 4.5 Hz, 1H), 7.42 (dt, J = 11.1, 7.4 Hz, 1H), 5.11 (s, 2H).

PREPARATION 26

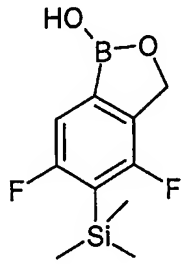
4,5,6-Trifluoro-3H-benzo[c][1,2]oxaborol-1-ol



Used crude without purification. ^{1}H NMR (d_6-DMSO) δ9.60 (bs, 1H), 7.55 (m, 1H), 5.10 (s, 2H).

PREPARATION 27

4,6-Difluoro-5-trimethylsilanyl-3H-benzo[c][1,2]oxaborol-1-ol



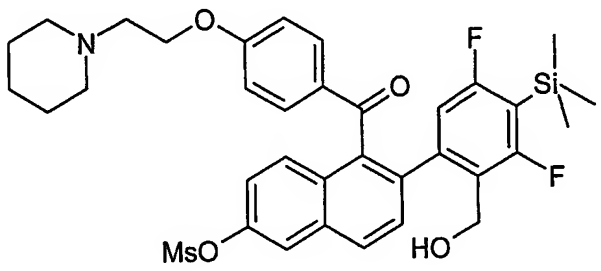
(100%). White solid. ^{1}H NMR (d_6-DMSO) δ9.51 (bs, 1H), 7.27 (d, J = 7.6 Hz, 1H), 5.02 (s, 2H), 0.34 (s, 9H).

General Method for the Suzuki Coupling

Charge a flask with trifluoro-methanesulfonic acid 6-methanesulfonyloxy-1-[4-(2-piperidin-1-yl-ethoxy)-benzoyl]-naphthalen-2-yl ester (1.0 eq.), hemiboronic ester from above (2.0 eq.) and $Pd(PPh_3)_4$ (0.1 eq.) and purge with nitrogen. Add degassed DME (0.1 M) and 2M Na_2CO_3 (10% of DME volume) to the flask and plunge into a 75 °C oil bath and stir overnight. Pour the reaction into saturated aqueous sodium bicarbonate and extract with methylene chloride. Wash the organic layer with water and dry with sodium sulfate. Filter and concentrate to an amber foam. Purify the crude material on silica gel (1% to 6% methanol in methylene chloride) to yield the title compound.

PREPARATION 28

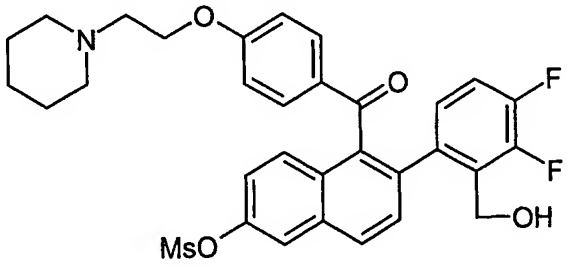
Methanesulfonic acid 6-(3,5-difluoro-2-hydroxymethyl-4-trimethylsilanyl-phenyl)-5-[4-(2-piperidin-1-yl-ethoxy)-benzoyl]-naphthalen-2-yl ester



(82%) Pale yellow foam. ^{1}H NMR ($CDCl_3$) δ7.99 (d, J = 8.4 Hz, 1H), 7.89 (d, J = 2.5 Hz, 1H), 7.70-7.52 (m, 3H), 7.49-7.38 (m, 2H), 7.35 (dd, J = 9.1, 2.3 Hz, 1H), 6.86 (d, J = 7.99 Hz, 2H), 6.44 (bs, 1H), 4.51 (bs, 1H), 4.14 (t, J = 6.0 Hz, 2H), 3.55 (s, 1H), 3.40 (s, 1H), 3.22 (s, 3H), 2.77 (t, J = 6.0 Hz, 2H), 2.49 (m, 4H), 1.59 (m, 4H), 1.45 (m, 2H), 0.35 (s, 9H). LCMS (25 to 95) R_t = 3.40 (94%) mass spectrum (apci) m/z= 668.4 (m+H).

PREPARATION 29

Methanesulfonic acid 6-(3,4-difluoro-2-hydroxymethyl-phenyl)-5-[4-(2-piperidin-1-yl-ethoxy)-benzoyl]-naphthalen-2-yl ester

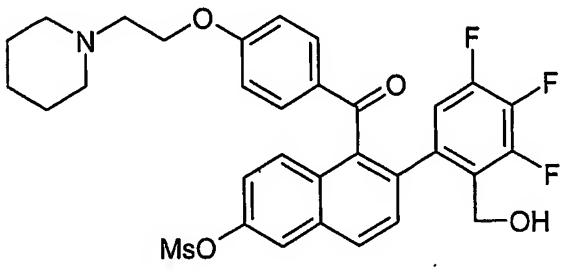


(96%) Tan foam. ^{1}H NMR ($CDCl_3$) δ8.00 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 2.5 Hz, 1H), 7-67-7.53 (m, 3H), 7.45-7.31 (m, 2H), 6.92-6.62 (m, 4H), 4.63 (bs, 1H), 4.13 (t, J = 5.6 Hz, 2H), 3.93 (bs, 1H), 3.23 (s, 3H), 2.77 (t, J = 6.0 Hz, 2H), 2.49 (bs, 4H), 1.59 (m,

4H), 1.44 (m, 2H). LCMS (25 to 95) R_t = 2.45 (86%) mass spectrum (apci) m/z= 596.3 (m+H).

PREPARATION 30

Methanesulfonic acid 5-[4-(2-piperidin-1-yl-ethoxy)-benzoyl]-6-(3,4,5-trifluoro-2-hydroxymethyl-phenyl)-naphthalen-2-yl ester



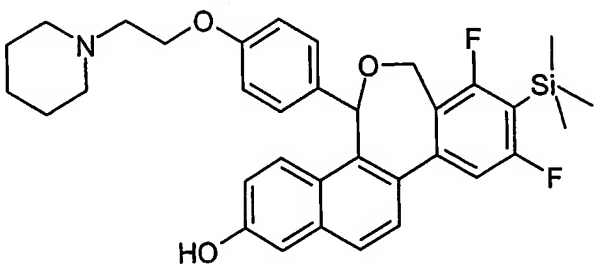
(94%) Tan foam. LCMS (25 to 95) R_t = 2.60 (50%) mass spectrum (apci) m/z= 614.3 (m+H).

General Method for the Reduction and Cyclization

Dissolve the product from the previous procedure (1.0 eq.) in dry THF (0.1M) and slowly add $LiAlH_4$ (1M in THF, 3.0 eq). After 30 minutes, slowly add saturated aqueous ammonium chloride until gas evolution ceases. Add sodium sulfate and stir for 1 hour. Filter and concentrate in vacuo. Dissolve the residue in dry THF (0.25M) and add HCl (4M in dioxane, 6.0 eq.). Stir at room temperature for 3 hours. Pour the reaction into water and extract with methylene chloride. Wash with saturated aqueous sodium bicarbonate. Dry over sodium sulfate, filter and concentrate in vacuo. Purify on silica gel (eluting with 2 to 5% methanol in methylene chloride) to yield the title compound.

Preparation 31

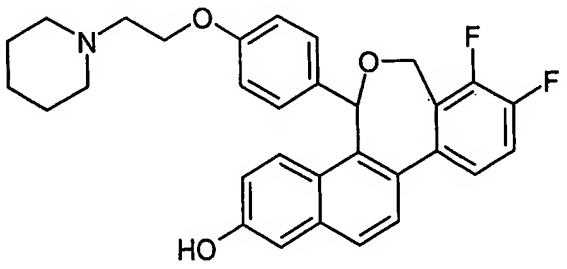
1,3-Difluoro-11-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-2-trimethylsilanyl-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol



(71%) ^{1}H NMR ($CDCl_3$) δ8.11 (d, J = 9.2 Hz, 1H), 7.7 (d, J = 8.6 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.17 (d, J = 2.7 Hz, 1H), 7.13 (dd, J = 9.2, 2.5 Hz, 1H), 6.81 (bs, 1H), 6.56 (m, 3H), 6.24 (d, J = 8.8 Hz, 2H), 5.04 (d, J = 12.1 Hz, 1H), 4.13 (dd, J = 11.9, 2.7 Hz, 1H), 3.97 (m, 2H), 2.77 (m, 2H), 2.58 (bs, 4H), 1.67 (m, 4H), 1.48 (m, 2H), 0.36 (s, 9H). LCMS (25 to 95) R_t = 4.14 (100%) mass spectrum (apci) m/z= 574.4 (m+H).

EXAMPLES 10 AND 11

1,2-Difluoro-11-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol hydrochloride salt



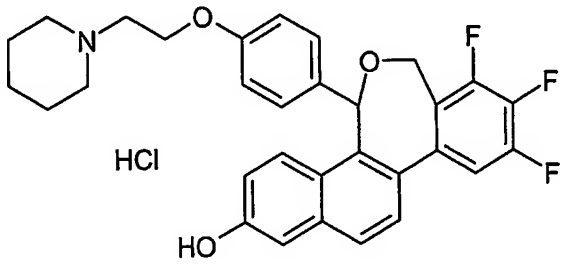
(56%) ^{1}H NMR (d_6-DMSO) δ 10.04 (s, 1H), 9.79 (bs, 1H), 8.35 (d, J = 9.4 Hz, 1H), 7.91 (d, J = 8.4 Hz, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.3 (m, 2H), 7.21 (dd, J = 9.3, 2.4 Hz, 1H), 7.11 (dd, J = 8.4, 4.5 Hz, 1H), 6.98 (s, 1H), 6.63 (m, 4H), 5.03 (d, J = 12.5 Hz, 1H), 4.17 (t, J = 5.0 Hz, 2H), 4.11 (dd, J = 12.3, 3.5 Hz, 1H), 3.37 (m, 4H), 2.92 (q, J =

10.1 Hz, 2H), 1.71 (m, 5H), 1.36 (m, 1H). Mass spectrum (apci) m/z=502.3 (M-HCl+H). HPLC (30 to 95) R_t (purity at 254 nm) = 1.73 min (100%).

Separate the enantiomers by chiral chromatography to yield Example 10 and Example 11.

EXAMPLES 12 AND 13

1,2,3-Trifluoro-11-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol hydrochloride salt

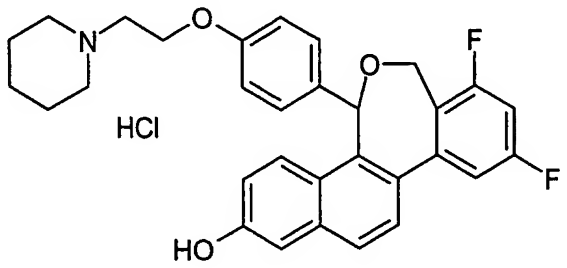


(20%) ^{1}H NMR (d_6-DMSO) δ10.11 (s, 1H), 9.91 (bs, 1H), 8.37 (d, J = 9.2 Hz, 1H), 7.93 (d, J = 8.4 Hz, 1H), 7.56 (d, J = 8.6 Hz, 1H), 7.31 (d, J = 2.5 Hz, 1H), 7.25 (m, 2H), 6.99 (s, 1H), 6.65 (m, 4H), 4.99 (d, J = 12.3 Hz, 1H), 4.19 (t, J = 5.1 Hz, 2H), 4.07 (dd, J = 11.9, 3.3 Hz, 1H), 3.39 (m, 4H), 2.92 (q, J = 10.1 Hz, 2H), 1.72 (m, 5H), 1.36 (m, 1H). Mass spectrum (apci) m/z=520.3 (M-HCl+H). HPLC (30 to 95) R_t (Purity at 254 nm) = 1.82 min (100%).

Separate the enantiomers by chiral chromatography to yield Example 12 and Example 13.

EXAMPLES 14 AND 15

1,3-Difluoro-11-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol hydrochloride salt

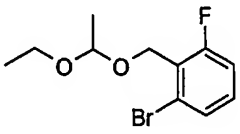


Dissolve 1,3-difluoro-11-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-2-trimethylsilanyl-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol (2.2g, 3.8 mmol) in acetonitrile (100 mL) and add tetrabutylammonium fluoride hydrate (2.0g, 7.6 mmol) and heat to 70°C for 4 hours. Cool the reaction to room temperature and partition between water and ethyl acetate. Extract the organic layer with water three times. Dry with sodium sulfate, filter and concentrate in vacuo. Dissolve the residue in methylene chloride (8 mL) and add HCl (2M in ether, 3.8 mL, 7.6 mmol). Add the solution slowly to vigorously stirred ether and collect the precipitate. Dry at 50 °C under vacuum over night to yield 1.9g (93%) of the title compound as a tan solid. ^{1}H NMR (d_6-DMSO) δ10.09 (s, 1H), 10.02 (bs, 1H), 8.36 (d, J = 9.2 Hz, 1H), 7.92 (d, J = 8.6 Hz, 1H), 7.57 (d, J = 8.6 Hz, 1H), 7.31 (d, J = 2.5 Hz, 1H), 7.22 (dd, J = 9.4, 2.7 Hz, 1H), 7.13 (td, J = 9.6, 2.3 Hz, 1H), 6.99 (m, 2H), 6.64 (m, 4H), 4.97 (d, J = 12.3 Hz, 1H), 4.19 (t, J = 5.2 Hz, 2H), 4.07 (d, J = 12.3 Hz, 1H), 3.37 (m, 4H), 2.91 (m, 2H), 1.70 (m, 5H), 1.57 (m, 1H). mass spectrum (apci) m/z=502.3 (M-HCl+H). HPLC (5 to 95) R_t (purity at 254 nm) = 2.76 min (>99%). Analysis calculated for $C_{31}H_{30}ClF_2NO_3 \bullet H_2O$: C, 66.96; H, 5.80; N, 2.52. Found: C, 67.00; H, 6.20; N, 2.83.

Separate the enantiomers by chiral chromatography to yield Example 14 and Example 15.

PREPARATION 32

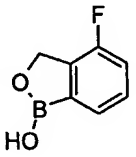
1-Bromo-2-(1-ethoxy-ethoxymethyl)-3-fluoro-benzene



(100%) ^{1}H NMR ($CDCl_3$) δ7.39 (d, J = 8.0 Hz, 1H), 7.16 (td, J = 8.2, 5.8 Hz, 1H), 7.04 (t, J = 8.7 Hz, 1H), 4.86 (q, J = 5.33 Hz, 1H), 4.80-4.64 (abx, J_{ab} = 10.5, J_{ax} = 2.3, J_{bx} = 2.1 Hz, 2H), 3.73 (m, 1H), 3.54 (m, 1H), 1.40 (d, J = 5.5 Hz, 3H), 1.22 (t, J = 7.0 Hz, 3H).

PREPARATION 33

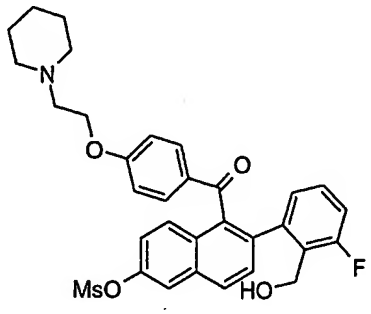
4-Fluoro-3H-benzo[c][1,2]oxaborol-1-ol



(85%) ^{1}H NMR (d_6-DMSO) δ 9.41 (bs, 1H), 7.57 (d, J = 7.2 Hz, 1H), 7.42 (td, J = 7.2, 4.6 Hz, 1H), 7.28 (ddd, J = 9.8, 8.0, 0.8 Hz, 1H), 5.08 (s, 2H).

PREPARATION 34

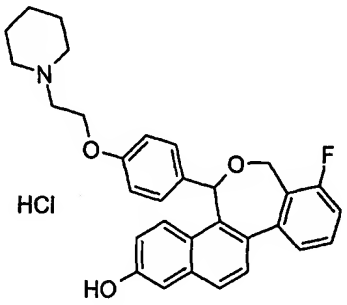
Methanesulfonic acid 6-(3-fluoro-2-hydroxymethyl-phenyl)-5-[4-(2-piperidin-1-yl-ethoxy)-benzoyl]-naphthalen-2-yl ester



(100%) ^{1}H NMR ($CDCl_3$) δ 7.99 (d, J = 8.6 Hz, 1H), 7.90 (d, J = 2.3 Hz, 1H), 7.70-7.40 (m, 4H), 7.35 (d, J = 9.6 Hz, 1H), 7.10-7.00 (m, 2H), 6.90-6.68 (m, 3H), 4.61 (bs, 1H), 4.25-4.10 (m, 3H), 3.75 (bs, 1H), 3.22 (s, 3H), 2.76 (t, J = 5.9 Hz, 2H), 2.49 (bs, 4H), 1.60 (m, 4H), 1.45 (m, 2H). LCMS (25 to 95) R_t = 2.19 (95%) mass spectrum (apci) m/z= 578.3 (m+H).

EXAMPLES 16 AND 17

1-Fluoro-11-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-11,13-dihydro-12-oxa-benzo[3,4]cyclohepta[1,2-a]naphthalen-8-ol



(70%) ^{1}H NMR (d_6-DMSO) δ 10.03 (s, 1H), 9.92 (bs, 1H), 8.33 (d, J = 9.2 Hz, 1H), 7.90 (d, J = 8.4 Hz, 1H), 7.55 (d, J = 8.6 Hz, 1H), 7.29 (d, J = 2.5 Hz, 1H), 7.26 (m, 1H), 7.20 (dd, J = 9.3, 2.7 Hz, 1H), 7.10 (m, 2H), 6.95 (bs, 1H), 6.65 (d, J = 8.6 Hz, 2H), 6.59 (d, J = 8.9 Hz, 2H), 5.00 (d, J = 11.7 Hz, 1H), 4.16 (t, J = 5.1 Hz, 2H), 4.12 (d, J = 11.9 Hz, 1H), 3.45-3.30 (m, 4H), 2.89 (m, 2H), 1.82-1.75 (m, 5H), 1.40-1.27 (m, 1H). Mass spectrum (apci) m/z=484.3 (M-HCl+H). HPLC (30 to 95) R_t (Purity at 254 nm) = 1.60 min (100%).

Separate the enantiomers by chiral chromatography to yield Example 16 and Example 17.

Biological Test Procedure

General Preparation Procedure

Competition binding assay is run in a buffer containing 50mM Hepes, pH 7.5, 1.5mM EDTA, 150mM NaCl, 10% glycerol, 1mg/ml ovalbumin and 5mM DTT, using 0.025 μCi per well ^{3}H-Estradiol(NEN #NET517 at 118 Ci/mmol, 1 mCi/ml), 10 ng/well ERAlpha or ERbeta receptor (PanVera). Competing compounds are added at 10 different concentrations. Non-specific binding is determined in the presence of 1μM of 17-B Estradiol. The binding reaction (140 μl) is incubated for 4 hours at room temperature, then 70 μl of cold DCC buffer is added to each reaction (DCC buffer contains per 50 ml

of assay buffer, 0.75g of charcoal (Sigma) and 0.25g of dextran (Pharmacia)). Plates are mixed 8 minutes on an orbital shaker at 4°C. Plates are then centrifuged at 3,000 rpm at 4°C for 10 minutes. An aliquot of 120µl of the mix is transferred to another 96-well, white flat bottom plate (Costar) and 175µl of Wallac Optiphase "Hisafe 3" scintillation fluid is added to each well. Plates are sealed and shaken vigorously on an orbital shaker. After an incubation of 2.5hrs, read plates in a Wallac Microbeta counter. The data is used to calculate an IC50 and % Inhibition at 10µM. The K_d for ^{3}H-Estradiol is determined by saturation binding to ER alpha and ER beta receptors. The IC_{50} values for compounds are converted to K_i using Cheng-Prusoff equation and the K_d determined by saturation binding assay.

Ishikawa human endometrial tumor cells are maintained in MEM (minimum essential medium, with Earle's salts and L-Glutamine, Gibco BRL, Gaithersburg, MD), supplemented with 10% fetal bovine serum (FBS) (V/V), (Gibco BRL). One day prior to assay, growth media is changed to assay medium, DMEM/F-12 (3:1) (Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12, 3:1 Mixture, phenol red-free, Gibco BRL) supplemented with 5% dextran coated charcoal stripped fetal bovine serum (DCC-FBS) (Hyclone, Logen, UT), L-Glutamine (2mM), MEM sodium pyruvate (1 mM), HEPES (N-[2-hydroxyethyl]piperazine-N' – [2-ethanesulfonic acid] 2 mM) all from Gibco BRL). After an overnight incubation, ishikawa cells are rinsed with Dulbecco's Phosphate Buffered Saline (1X) (D-PBS) without Ca^{+2} and Mg^{+2} (Gibco BRL), and trypsinized by a 3 minute incubation with 0.25% Trypsin/EDTA, phenol red-free (Gibco BRL). Cells are resuspended in assay medium and adjusted to 250,000 cells/ml. Approximately 25,000 cells in a 100ul media are added to flat-bottom 96 wells microculture plates (Costar 3596) and incubated at 37°C in a 5% CO_2 humidified incubator for 24 hours. The next day, serial dilutions of compounds are prepared in assay medium (at 6 times the final concentration in the assay). The assay is run in dual mode, agonist and antagonist modes. For the agonist mode, plates receive 25 µl/well of assay medium followed by 25 µl/well of diluted compounds (at 6x the final concentrations). For the antagonist mode, plates receive 25 µl/well of 6 nM E_2 (β-Estradiol, Sigma, St. Louis, MO) followed by 25 µl/well of diluted compounds (at 6x the final concentrations). After an additional 48-hour incubation at 37°C in a 5% CO_2 humidified incubator, media is

aspirated from wells and 100 µl fresh assay medium is added to each microculture. Serial dilutions of compounds are prepared and added to the cells as described above. After an additional 72 hour incubation at 37°C in a 5% CO_2 humidified incubator, the assay is quenched by removing media and rinsing plates twice in Dulbecco's Phosphate Buffered Saline (1X) (D-PBS) (Gibco BRL). The plates are dried for 5 min and frozen at -70°C for at least 1 hour. The plates are then removed from the freezer and allowed to thaw at room temperature. To each well, 100 µl of 1-Step™ PNPP (Pierce Chemical Company, Rockford, IL) is added. After a 20-minute incubation, plates are read on a spectophotometer at 405nm. The data is fitted to a linear interpolation to derive EC50 (for agonist mode) or IC50 (for antagonist mode) values. For the agonist mode, a % efficacy for each compound is calculated versus the response to tamoxifen. For the antagonist mode, a % efficacy for each compound is calculated versus E2 (1nM) alone.

MCF-7 breast adenocarcinoma cells (ATCC HTB 22) are maintained in MEM (minimal essential medium, phenol red-free, Gibco BRL) supplemented with 10% fetal bovine serum (FBS) (V/V), L-glutamine (2 mM), sodium pyruvate (1 mM), HEPES ((N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]10 mM}, non-essential amino acids(0.1mM)and Penicillin Streptomycin(1X). Seven days prior to assay, MCF-7 cells are switched to assay media which is the same as maintenance medium except supplemented with 10% dextran-coated charcoal-stripped fetal bovine serum (DCC-FBS) assay medium in place of 10% FBS. MCF-7 cells are removed from flasks using 10X Trypsin EDTA (phenol red free, Gibco BRL) and diluted to 1X in (Ca++/Mg++ free HBSS (phenol red-free). Cells are adjusted to 80,000 cells/ml in assay medium. Approximately 8,000 cells (100 µl) are added to each well in 96 well Cytostar T scintillation plates (Amersham) and incubated at 37°C in a 5% CO_2 humidified incubator for 24 hours to allow cell adherence and equilibration after transfer. Serial dilutions of drugs are prepared in assay medium at 4x the final desired concentration). A 50 µl aliquot of drug dilutions (at 4x the final assay concentration) is transferred to duplicate wells followed by 50 µl assay medium for the agonist mode or 50 µl of 40pM of E2 for the antagonist mode to a final volume of 200 µl. For each of the agonist plates, a basal level (media) and a maximum stimulated level (with 1µM E2) is determined. For each of the antagonist plates, a basal level (media) and a E2 (10pM) alone control is determined.

After an additional 48 hours at 37°C in a 5% CO_2 humidified incubator, 20µl of assay medium containing 0.01 µCi of ^{14}C-thymidine (52 mCi/mmol, 50 µCi/ul, Amersham) is added to each well. The plates are incubated overnight in the same incubator and then counted on the Wallac Microbeta counter. The data is averaged to calculate an IC50 and % inhibition @ 1µM for the antagonist mode. For the agonist mode, an EC50 and percent of maximum E2 stimulation and concentration of maximum stimulation is calculated.

TABLE

	ER binding		MCF-7	Ishikawa		
Cmpnd (Ex. No.)	K_i (ERα) (nM)	K_i (ERβ) (nM)	MCF-7 IC50 (nM)	Ishikawa EC50 (nM)	Agonist % Eff	IC50
1	240	N.D.	N.D.	300	31	N.D.
2	2.9	19	36	2.8	41	270
3	140	430	480	260	21	340
4*	2.6	4.0	2.4	1.4	17	36
6	58	120	160	54	38	740
7	0.7	1.7	0.4	N.D.	2.6	2.2
8	0.7	1.4	0.4	N.D.	8.8	4.3
9	62	89	68	N.D.	11	350

* signifies the trifluoroacetic acid (TFA) salt

General Rat Preparation Procedure

Seventy-five day old (unless otherwise indicated) female Sprague Dawley rats (weight range of 200 to 225g) are obtained from Charles River Laboratories (Portage, MI). The animals are either bilaterally ovariectomized (OVX) or exposed to a Sham surgical procedure at Charles River Laboratories, and then shipped after one week. Upon arrival, they are housed in metal hanging cages in groups of 3 or 4 per cage and have *ad libitum* access to food (calcium content approximately 0.5%) and water for one week. Room temperature is maintained at 22.2° ± 1.7°C with a minimum relative humidity of 40%. The photoperiod in the room was 12 hours light and 12 hours dark.

Dosing Regimen Tissue Collection: After a one week acclimation period (therefore, two weeks post-OVX) daily dosing with a compound of formula (I) ("F-I") is initiated. 17α-ethynyl estradiol or F-I is given orally, unless otherwise stated, as a suspension in 1% carboxymethylcellulose or dissolved in 20% cyclodextrin. Animals are dosed daily for 4 days. Following the dosing regimen, animals are weighed and anesthetized with a ketamine: Xylazine (2:1, v:v) mixture and a blood sample is collected by cardiac puncture. The animals are then sacrificed by asphyxiation with CO_2, the uterus is removed through a midline incision, and a wet uterine weight is determined. 17α-ethynyl estradiol is obtained from Sigma Chemical Co., St. Louis, MO.

Cardiovascular Disease/Hyperlipidemia

The blood samples from above are allowed to clot at room temperature for 2 hours, and serum is obtained following centrifugation for 10 minutes at 3000 rpm. Serum cholesterol is determined using a Boehringer Mannheim Diagnostics high performance cholesterol assay. Briefly the cholesterol is oxidized to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide is then reacted with phenol and 4-aminophenazone in the presence of peroxidase to produce a p-quinone imine dye, which is read spectrophotemetrically at 500 nm. Cholesterol concentration is then calculated against a standard curve. The entire assay is automated using a Biomek Automated Workstation.

Uterine Eosinophil Peroxidase (EPO) Assay

The uteri from above are kept at 4°C until time of enzymatic analysis. The uteri are then homogenized in 50 volumes of 50 mM Tris buffer (pH - 8.0) containing 0.005% Triton X-100. Upon addition of 0.01% hydrogen peroxide and 10 mM O-phenylenediamine (final concentrations) in Tris buffer, increase in absorbance is monitored for one minute at 450 nm. The presence of eosonophils in the uterus is an indication of estrogenic activity of a compound. The maximal velocity of a 15 second interval is determined over the initial, linear portion of the reaction curve.

Inhibition of Bone Loss (Osteoporosis) Test Procedure

Following the general preparation procedure described above, the rats are treated daily for thirty-five days (6 rats per treatment group) and sacrificed by carbon dioxide

asphyxiation on the 36th day. The thirty-five day time period is sufficient to allow maximal reduction in bone density, measured as described herein. At the time of sacrifice, the uteri are removed, dissected free of extraneous tissue, and the fluid contents are expelled before determination of wet weight in order to confirm estrogen deficiency associated with complete ovariectomy. Uterine weight is routinely reduced about 75% in response to ovariectomy. The uteri are then placed in 10% neutral buffered formalin to allow for subsequent histological analysis.

The right femurs are excised and digitilized X-rays generated and analyzed by an image analysis program (NIH image) at the distal metaphysis. The proximal aspect of the tibiae from these animals are also scanned by quantitative computed tomography. In accordance with the above procedures, F-I or ethynyl estradiol (EE_2) in 20% hydroxypropyl β-cyclodextrin are orally administered to test animals. F-I is also useful in combination with estrogen or progestin.

Uterine Fibrosis Test Procedures

Test 1: Between 3 and 20 women having uterine fibrosis are administered F-I. The amount of compound administered is from 0.1 to 1000 mg/day, and the period of administration is 3 months. The women are observed during the period of administration, and up to 3 months after discontinuance of administration, for effects on uterine fibrosis.

Test 2: The same procedure is used as in Test 1, except the period of administration is 6 months.

Test 3: The same procedure is used as in Test 1, except the period of administration is 1 year.

Test 4: Prolonged estrogen stimulation is used to induce leiomyomata in sexually mature female guinea pigs. Animals are dosed with estradiol 3-5 times per week by injection for 2-4 months or until tumors arise. Treatment consisting of F-I or vehicle is administered daily for 3-16 weeks and then animals are sacrificed and the uteri harvested and analyzed for tumor regression.

Test 5: Tissue from human leiomyomas are implanted into the peritoneal cavity and/or uterine myometrium of sexually mature, castrated, female, nude mice. Exogenous estrogen is supplied to induce growth of the explanted tissue. In some cases, the harvested tumor cells are cultured *in vitro* prior to implantation. Treatment consisting of F-I or vehicle is supplied by gastric lavage on a daily basis for 3-16 weeks and implants are removed and measured for growth or regression. At the time of sacrifice, the uteri are harvested to assess the status of the organ.

Test 6: Tissue from human uterine fibroid tumors is harvested and maintained, *in vitro*, as primary non-transformed cultures. Surgical specimens are pushed through a sterile mesh or sieve, or alternately teased apart from surrounding tissue to produce a single cell suspension. Cells are maintained in media containing 10% serum and antibiotic. Rates of growth in the presence and absence of estrogen are determined. Cells are assayed for their ability to produce complement component C3 and their response to growth factors and growth hormone. *In vitro* cultures are assessed for their proliferative response following treatment with progestins, GnRH, F-I, and vehicle. Levels of steroid hormone receptors are assessed weekly to determine whether important cell characteristics are maintained *in vitro*. Tissue from 5-25 patients is utilized.

Test 7: F-I's ability to inhibit estrogen-stimulated proliferation of leiomyoma-derived ELT cell lines is measured substantially as described in Fuchs-Young, et al., "Inhibition of Estrogen-Stimulated Growth of Uterine Leiomyomas by Selective Estrogen Receptor Modulators", Mol. Car., 17(3):151-159 (1996), the teachings of which are herein incorporated by reference.

Endometriosis Test Procedures

Test 1: Twelve to thirty adult CD strain female rats are used as test animals. They are divided into three groups of equal numbers. The estrous cycle of all animals is monitored. On the day of proestrus, surgery is performed on each female. Females in each group have the left uterine horn removed, sectioned into small squares, and the squares are loosely sutured at various sites adjacent to the mesenteric blood flow. In addition, females in Group 2 have the ovaries removed. On the day following surgery, animals in Groups 1 and 2 receive intraperitoneal injections of water for 14 days whereas

animals in Group 3 receive intraperitoneal injections of 1.0 mg of F-I per kilogram of body weight for the same duration. Following 14 days of treatment, each female is sacrificed and the endometrial explants, adrenals, remaining uterus, and ovaries, where applicable, are removed and prepared for histological examination. The ovaries and adrenals are weighed.

Test 2: Twelve to thirty adult CD strain female rats are used as test animals. They are divided into two equal groups. The estrous cycle of all animals is monitored. On the day of proestrus, surgery is performed on each female. Females in each group have the left uterine horn removed, sectioned into small squares, and the squares are loosely sutured at various sites adjacent to the mesenteric blood flow. Approximately 50 days following surgery, animals assigned to Group 1 receive intraperitoneal injections of water for 21 days whereas animals in Group 2 receive intraperitoneal injections of 1.0 mg of F-I per kilogram of body weight for the same duration. Following 21 days of treatment, each female is sacrificed and the endometrial explants and adrenals are removed and weighed. The explants are measured as an indication of growth. Estrous cycles are monitored.

Test 3: Autographs of endometrial tissue are used to induce endometriosis in rats and/or rabbits. Female animals at reproductive maturity undergo bilateral oophorectomy, and estrogen is supplied exogenously thus providing a specific and constant level of hormone. Autologous endometrial tissue is implanted in the peritoneum of 5-150 animals and estrogen supplied to induce growth of the explanted tissue. Treatment consisting of a compound of the present invention is supplied by gastric lavage on a daily basis for 3-16 weeks, and implants are removed and measured for growth or regression. At the time of sacrifice, the intact horn of the uterus is harvested to assess status of endometrium.

Test 4: Tissue from human endometrial lesions is implanted into the peritoneum of sexually mature, castrated, female, nude mice. Exogenous estrogen is supplied to induce growth of the explanted tissue. In some cases, the harvested endometrial cells are cultured *in vitro* prior to implantation. Treatment consisting of F-I supplied by gastric lavage on a daily basis for 3-16 weeks, and implants are removed and measured for

growth or regression. At the time of sacrifice, the uteri are harvested to assess the status of the intact endometrium.

Test 5: Tissue from human endometrial lesions is harvested and maintained *in vitro* as primary non-transformed cultures. Surgical specimens are pushed through a sterile mesh or sieve, or alternately teased apart from surrounding tissue to produce a single cell suspension. Cells are maintained in media containing 10% serum and antibiotic. Rates of growth in the presence and absence of estrogen are determined. Cells are assayed for their ability to produce complement component C3 and their response to growth factors and growth hormone. *In vitro* cultures are assessed for their proliferative response following treatment with progestins, GnRH, F-I, and vehicle. Levels of steroid hormone receptors are assessed weekly to determine whether important cell characteristics are maintained *in vitro*. Tissue from 5-25 patients is utilized.

Use of Formula (I) Compound in Conjunction with Estrogen

Peri- and post-menopausal women often undergo hormone replacement therapy (HRT) to combat negative consequences associated with the drop in circulating endogenous estrogen, *e.g.*, to treat hot flashes. However, HRT has been associated with increased risks of certain cancers including uterine and breast cancer. F-I may be employed in conjunction with HRT to inhibit these risks.

Prevention of Breast Cancer

This invention also relates to the administration of F-I to a recipient who is at risk of developing *de novo* breast cancer. The term "*de novo*", as used herein, means the lack of transformation or metamorphosis of normal breast cells to cancerous or malignant cells in the first instance. Such a transformation may occur in stages in the same or daughter cells via an evolutionary process or may occur in a single, pivotal event. This *de novo* process is in contrast to the metastasis, colonization, or spreading of already transformed or malignant cells from the primary tumor site to new locations.

A person who is at no particular risk of developing breast cancer is one who may develop *de novo* breast cancer, has no evidence or suspicion of the potential of the disease above normal risk, and who has never had a diagnosis of having the disease. The greatest

risk factor contributing to the development of breast carcinoma is a personal history of suffering from the disease, or an earlier occurrence of the disease, even if it is in remission with no evidence of its presence. Another risk factor is family history of the disease.

Induction of mammary tumors in rats by administration of the carcinogen N-nitroso-N-methylurea is a well-accepted animal model for the study of breast cancer and has been found suitable for analyzing the effect of chemopreventive agents.

In two separate studies, 55-day old female Sprague-Dawley rats are given an intravenous (Study 1) or intraperitoneal (Study 2) dose of 50 mg of N-nitroso-N-methylurea per kilogram of body weight one week prior to feeding *ad libitum* a diet into which varying amounts of F-I, (Z)-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-*N,N*-dimethylethanamine base (tamoxifen base), or control are blended.

In Study 1, the dietary doses of 60 mg/kg of diet and 20 mg/kg of diet translates into roughly comparable doses of 3 and 1 mg/kg of body weight for the test animals.

In Study 2, the dietary doses of 20, 6, 2, and 0.6 mg/kg of diet translates roughly into comparable doses of 1, 0.3, 0.1 and 0.03 mg/kg of body weight for the test animals.

Rats are observed for evidence of toxicity and are weighed and palpated for tumor formation once a week. The animals are sacrificed after thirteen weeks (Study 1) or eighteen weeks (Study 2) and tumors are confirmed and weighed at autopsy.

Therapeutic Methods of Use and Dosages

The present invention also provides a method of inhibiting a disease associated with estrogen deprivation and a method for inhibiting a disease associated with an aberrant physiological response to endogenous estrogen which comprises the aforementioned method using compounds of Formula I and optionally comprises administering to a patient an effective amount of estrogen or progestin. These treatments are particularly useful for treating osteoporosis and lowering serum cholesterol because the patient will receive the benefits of each pharmaceutical agent while the compounds of the present invention would inhibit undesirable side-effects of estrogen and progestin. Activity of these combination treatments in any of the post-menopausal tests, infra, indicates that the combination treatments are useful for alleviating the symptoms of post-menopausal symptoms in women.

Various forms of estrogen and progestin are commercially available. Estrogen-based agents include, for example, ethynyl estrogen (0.01 - 0.03 mg/day), mestranol (0.05 - 0.15 mg/day), and conjugated estrogenic hormones such as Premarin® (Wyeth-Ayerst; 0.3 - 2.5 mg/day). Progestin-based agents include, for example, medroxyprogesterone such as Provera® (Upjohn; 2.5 -10 mg/day), norethylnodrel (1.0 - 10.0 mg/day), and nonethindrone (0.5 - 2.0 mg/day). A preferred estrogen-based compound is Premarin®, and norethylnodrel and norethindrone are preferred progestin-based agents.

The method of administration of each estrogen- and progestin-based agent is consistent with that which is known in the art. For the majority of the methods of the present invention, compounds of Formula I are administered continuously, from 1 to 3 times daily. However, cyclical therapy may especially be useful in the treatment of endometriosis or may be used acutely during painful attacks of the disease. In the case of restenosis, therapy may be limited to short (1-6 months) intervals following medical procedures such as angioplasty.

As used herein, the term "patient" refers to a warm-blooded animal or mammal which is in need of inhibiting a disease associated with estrogen deprivation or in need of inhibiting a disease associated with an aberrant physiological response to endogenous estrogen. It is understood that guinea pigs, dogs, cats, rats, mice, hamsters, and primates, including humans, are examples of patients within the scope of the meaning of the term. Preferred patients include humans. Most preferred patients include postmenopausal female humans.

As used herein, the term "inhibit" is defined to include its generally accepted meaning which includes preventing, prohibiting, restraining, and slowing, stopping or reversing progression, or severity, and holding in check and/or treating existing characteristics. The present method includes both medical therapeutic and/or prophylactic treatment, as appropriate.

The term "estrogen deprivation" is meant to imply the condition where the optimal level of estrogen is absent. This level varies from one tissue to another depending on the function of the tissue. Thus, in some cases, estrogen deprivation may be the total absence of estrogen, whereas in other cases, deprivation may involve estrogen levels which are too low for proper tissue function. In human women, the two most common causes of estrogen deprivation are menopause and ovariectomy, although other conditions can be

causative. Estrogen deprivation can lead to conditions including osteoporosis and cardiovascular effects such as hyperlipidemia, proliferation of aortal smooth muscle cells (restenosis), decrease in nitric oxide production (hypertension) and decrease in production of the enzyme PAI-1 (Plasminogen Activator Inhibitor-1), i.e. thrombosis.

Reduction or amelioration of other pathologies associated with menopause such as urinary incontinence, vaginal dryness, increase in the incidence of auto-immune disease, and loss of skin tone, may also be achieved by administering compounds of Formula I.

In addition to their usefulness in treating conditions associated with estrogen deprivation following menopause, the compounds of the present invention are also useful in the treatment of disease states associated with inappropriate response to endogenous estrogen in tissues both prior to and subsequent to menopause.

One example of a pathological condition associated with abnormal cellular responses to endogenous estrogen in tissues is estrogen dependent breast cancer. Estrogen dependent breast tumor cells proliferate in the presence of estrogen and the treatment of this disease has been to stop all action of estrogen on these cells.

Another estrogen dependent pathology is uterine fibrosis (uterine fibroid disease). Essentially, uterine fibrosis is a condition where there is a deposition of fibroid tissue on the wall of the uterus. This condition is a cause of dysmenorrhea and infertility in women. The exact cause of this condition is poorly understood but evidence suggests that it is an inappropriate response of fibroid tissue to estrogen. The most common treatment of uterine fibrosis involves surgical procedures both costly and sometimes a source of complications such as the formation of abdominal adhesions and infections.

Yet another disease in this category is endometriosis, a condition of severe dysmenorrhea, which is accompanied by severe pain, bleeding into the endometrial masses or peritoneal cavity and often leads to infertility. The cause of the symptoms of this condition appear to be ectopic endometrial growths located in inappropriate tissues which respond inappropriately to hormonal control.

As used herein, the term "therapeutically effective amount" means an amount of compound of the present invention which is capable of alleviating the symptoms of the various pathological conditions herein described. The specific dose of a compound administered according to this invention will, of course, be determined by the particular circumstances surrounding the case including, for example, the compound administered,

the route of administration, the state of being of the patient, and the pathological condition being treated. A typical daily dose for human use will contain a nontoxic dosage level of from about 0.1 mg to about 600 mg/day of a compound of the present invention administered once to three times per day. Preferred daily doses generally will be from about 1 mg to about 300 mg/day. Most preferred doses generally will be from about 20 mg to about 100 mg one to three times per day.

The compounds of this invention can be administered by a variety of routes including oral, rectal, transdermal, subucutaneus, intravenous, intramuscular, and intranasal. These compounds preferably are formulated prior to administration, the selection of which will be decided by the attending physician. Thus, another aspect of the present invention is a pharmaceutical composition comprising an effective amount of a compound of Formula I, or a pharmaceutically acceptable salt thereof, optionally containing an effective amount of estrogen or progestin, and a pharmaceutically acceptable carrier, diluent, or excipient.

The total active ingredients in such formulations comprises from 0.1% to 99.9% by weight of the formulation. By "pharmaceutically acceptable" it is meant the carrier, diluent, excipients and salt must be compatible with the other ingredients of the formulation, and not deleterious to the recipient thereof.

Pharmaceutical formulations of the present invention can be prepared by procedures known in the art using well known and readily available ingredients. For example, the compounds of formula I, with or without an estrogen or progestin compound, can be formulated with common excipients, diluents, or carriers, and formed into tablets, capsules, suspensions, powders, and the like. Examples of excipients, diluents, and carriers that are suitable for such formulations include the following: fillers and extenders such as starch, sugars, mannitol, and silicic derivatives; binding agents such as carboxymethyl cellulose and other cellulose derivatives, alginates, gelatin, and polyvinyl-pyrrolidone; moisturizing agents such as glycerol; disintegrating agents such as calcium carbonate and sodium bicarbonate; agents for retarding dissolution such as paraffin; resorption accelerators such as quaternary ammonium compounds; surface active agents such as cetyl alcohol, glycerol monostearate; adsorptive carriers such as kaolin and bentonite; and lubricants such as talc, calcium and magnesium stearate, and solid polyethyl glycols.

The compounds also can be formulated as elixirs or solutions for convenient oral administration or as solutions appropriate for parenteral administration, for example, by intramuscular, subcutaneous or intravenous routes. Additionally, the compounds are well suited to formulation as sustained release dosage forms and the like. The formulations can be so constituted that they release the active ingredient only or preferably in a particular physiological location, possibly over a period of time. The coatings, envelopes, and protective matrices may be made, for example, from polymeric substances or waxes.

Compounds of formula I, alone or in combination with a pharmaceutical agent of the present invention, generally will be administered in a convenient formulation.

The compounds of the present invention may contain one or more asymmetric centers, and therefore can exist as a mixture of isomers, or as individual isomers. Either a mixture or individual isomers will be useful for the purposes of the present invention, and can be so employed.